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1. Your reference 9482 GB/JSvn

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ICH Productions Limited
30 Guildford Street
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England

Patents ADP number *(if you know it)* 7273816001

If the applicant is a corporate body, give the country/state of its incorporation: England

4. Title of the invention

PEPTIDE LIGANDS

5. Name of your agent *(if you have one)*

Abel & Imray

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

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WC1R 4PQ
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12. Name and daytime telephone number of person to contact in the United Kingdom — Judith Silveston 020 7242 9984

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PEPTIDE LIGANDS

FIELD OF THE INVENTION

The present invention relates to peptide ligands that bind to dendritic cells, and their use *inter* alia in vector systems having improved efficiency of gene transfer.

BACKGROUND OF THE INVENTION

Gene therapy and gene vaccination are techniques that offer interesting possibilities for the treatment and/or prophylaxis of a variety of conditions, as does anti-sense therapy. Such techniques require the introduction of a nucleic acid of interest into target cells. The ability to transfer sufficient nucleic acid to specific target cells remains one of the main limitations to the development of gene therapy, anti-sense therapy and gene vaccination. Both viral and non-viral nucleic acid delivery systems have been proposed. The nucleic acid is generally DNA, but in some cases RNA is used.

The term "gene" is used somewhat loosely in the context of gene vaccination and, especially, gene therapy. While, initially, the term "gene" in those contexts was used to denote the coding sequence of a protein, the term is now used in a general sense to refer to a useful nucleic acid. Examples of nucleic acids that can be used in gene therapy and/or in gene vaccination include the coding sequence of a protein and the cDNA copy and genomic version thereof, the latter including introns as well as exons, and also the regulatory upstream and downstream sequences. Other useful nucleic acids include sequences involved in repairing genes and in homologous recombination. These can be molecules such as RNA/DNA chimeras (Bandyopadhyay et al., 1999; Cole-Strauss et al., 1996; Kren et al., 1998; Yoon et al., 1996) or DNA oligonucleotides (Goncz et al., 1998). A useful nucleic acid

can be a short sequence contained in a plasmid, or another large nucleic acid encoding an enzyme that mediates integration of plasmids or nucleic acids, for example, the ϕC31 phage attB/integrase system (Groth et al., 2000; Olivares et al., 2001; Stoll et al., 2002; Thyagarajan et al., 2000; Thyagarajan et al., 2001)and the "Sleeping Beauty" transposon/transposase system (Yant et al., 2000).

DNA oligonucleotides can be delivered for purposes of antisense regulation (Bachmann et al., 1998; Knudsen and Nielsen, 1997; Mannion et al., 1998; Woolf et al., 1995) or as transcription factor decoys (Ehsan et al., 2001; Ehsan et al., 2002; Mann et al., 1999; Morishita et al., 1995). CpG-rich oligonucleotide sequences may be useful as adjuvants to boost vaccine responses (Krieg et al., 1995).

Another important new class of nucleic acids that can be used in gene therapy includes double-stranded RNA 20-30 nt in length known as small interfering RNA molecules (siRNA). RNA interference in mammalian cells has emerged in the last two or three years as an important new approach to the regulation of gene expression, with a high degree of specificity (reviewed Shi 2003). siRNA) molecules target homologous regions of mRNA then activate a conserved pathway that leads to degradation of the mRNA target. The precise mechanism of action of siRNA is under intense investigation but it is clear that the application of siRNA to mammalian cells has the potential to revolutionize the field of functional genomics. The ability to simply, effectively, and specifically down-regulate the expression of genes in mammalian cells holds enormous scientific, commercial, and therapeutic potential.

Currently there is no way to predict an effective siRNA target so screening of numerous sequences is performed and

numerous potential molecules may have to be screened. Such screening is most conveniently performed with chemically synthesised siRNA molecules delivered by non-viral vectors. Improved vectors for siRNA transfection would thus provide benefits of cost-effectiveness as well as greater functionality. *In vivo* use of siRNA molecules in animal models is at a much earlier stage of development but there, too, the potential is enormous.

There are two main modes of transfer of nucleic acid into cells, namely, transfer of naked nucleic acid, and vector-mediated transfer. Non-viral or synthetic vectors fall into three main groups, lipid vectors (lipoplex vectors), vectors comprising other non-lipidic cationic polymers including peptides, dendrimers, and polyethylenimine (PEI) (polyplex vectors), and vectors comprising both cationic polymers and lipids (lipopolyplex vectors) (Felgner et al., 1997). Targeted vectors include viral vectors and receptor-targeted synthetic vectors.

Viral vectors commonly used for gene transfer and hence gene therapy and gene vaccination include genetically engineered, replication-defective derivatives of retrovirus, lentivirus, adenovirus, adeno-associated virus (AAV), and herpes simplex virus (HSV). They generally exhibit high efficiency of gene transfer *in vitro* and in some cases, in vivo, in cell types for the which the virus is trophic, i.e., which contain the native receptors. However, gene transfer is poor in cell types that do not contain a native receptor for the virus. Additionally retroviruses are restricted to transducing cells that are dividing rapidly. Furthermore, most viral vectors are restricted in their packaging capacity for nucleic acids, for example, AAV 5kb; adenovirus 7-8 kb; 35 kb for helper-dependent adenovirus; and retrovirus 10kb. HSV can package much larger constructs, up to 135-kb (Wade-Martins et al.,

2003). Methods of production of replication deficient viral vectors are generally prolonged procedures and in some cases yields of virus are low.

Receptor-mediated gene delivery is a non-viral method of gene transfer that exploits the physiological cellular process of receptor-mediated endocytosis to internalise the nucleic acid. Examples include vectors targeted against insulin receptors, see for example, Rosenkranz et al Experimental Cell Research 199, 323-329 (1992), asialoglycoprotein receptors, see for example, Wu & Wu, Journal of Biological Chemistry 262, 4429-4432 (1987), Chowdhury et al Journal of Biological Chemistry 268, 11265-11271 (1993), and transferrin receptors, see for example, Ciriel et al, Proc. Natl. Acad. Sci. USA 88, 8850-8854 (1991). Further examples of vectors include monoclonal antibodies that target receptors on neuroblastoma cells (Yano et al, 2000), folate conjugated to liposomes (Reddy & Low 2000, Reddy et al. 1999), galactose for targeting liver cells (Han et al. 1999 Bettinger et al. 1999) and asialogylcoprotein, also for liver cells (Wu et al. 1991).

Receptor-mediated non-viral vectors have several advantages over viral vectors. In particular, they lack pathogenicity; they allow targeted gene delivery to specific cell types and they are not restricted in the size of nucleic acid molecules that can be packaged. Gene expression is achieved only if the nucleic acid component of the transfection complex is released intact from the endosome to the cytoplasm and then crosses the nuclear membrane to access the nuclear transcription machinery. However, transfection efficiency is generally poor relative to viral vectors owing to endosomal degradation of the nucleic acid component, failure of the nucleic acid to enter the nucleus and the exclusion of aggregates larger than about 150nm from clathrin coated

vesicles.

Desirable properties of targeting ligands for vectors are that they should bind to cell-surface receptors with high affinity and specificity and mediate efficient vector internalisation. Short peptides have particular advantages as targeting ligands since they are straightforward to synthesise in high purity and, importantly for *in vivo* use, they have low immunogenic potential.

WO 98/54347 discloses a mixture comprising an integrin-binding component, a polycationic nucleic acid-binding component, and a lipid component, and also discloses a transfection complex comprising
(i) a nucleic acid, especially a nucleic acid encoding a sequence of interest,
(ii) an integrin-binding component,
(iii) a polycationic nucleic acid-binding component, and
(iv) a lipid component.
The transfection complex is primarily an integrin-mediated transfection vector.

It is considered that the components described in WO 98/54347 associate electrostatically to form the vector complex, the vector being of the lipopolyplex type. The vector complexes of WO 98/54347 are found to transfect a range of cell lines and primary cell cultures with high efficiency, with integrin specificity and with low toxicity. For example, vascular smooth muscle cells are transfected with 50% efficiency, endothelial cells with 30% efficiency and haematopoietic cells with 10% efficiency. Furthermore, *in vivo* transfection of bronchial epithelium of rat lung and pig lung with an efficiency comparable with that of an adenoviral vector has been demonstrated.

Vectors that utilise integrin receptors to mediate gene transfer have the advantage that they target a large number of different types of cells in the body as integrin receptors are relatively widespread. In some circumstances, for example, in *in vivo* treatment, however, it may be preferable to target recipient cells more specifically.

The dendritic cell is the most potent antigen presenting cell of the immune system and is the only antigen presenting cell capable of stimulating naïve T cell clones, which requires not only recognition of antigenic peptide presented by MHC but also binding costimulatory molecules. The main function of immature dendritic cells is antigen uptake from the surrounding environment. Maturation occurs upon exposure of the cell to danger signals and the function of the cell changes from antigen uptake to peptide presentation on the MHC molecules, combined with trafficking of the dendritic cell to the lymph nodes. Full maturation occurs when the dendritic cells are within the lymph nodes and it is thought that injection or other administration of mature dendritic cells may lead to impairment of homing of the cells.

Transduction of immature dendritic cells also allows for the transfection of cytokine genes to increase the immune response, whilst also allowing for presentation of peptides taken up from the environment where they have been injected.

Transfection efficiencies to immature dendritic cells using nonviral vectors have been poor, partly due to toxicity. Transfection efficiencies to immature dendritic cells using adenovirus have required high titres of virus, due at least in part to the paucity of the primary adenoviral receptor, the Coxsackie-Adenovirus Receptor (CAR) on the immature dendritic cell surface. Using nonviral vectors, efficiencies have been increased by altering the lipid used. Various

strategies have been attempted to increase adenoviral transduction of dendritic cells, including targeting using bispecific antibody fragments (scFv) (Brandao 2003). The use of less adenovirus and a shorter transduction time would be preferable for *ex vivo* transduction for clinical purposes.

It is an object of the present invention to provide improved vector complexes with enhanced cell targeting properties. The present invention is based on the development of synthetic, targeting non-viral vector complexes that carry a ligand that is more cell-type selective than the ligands of the prior art.

In the development of effective targeting vectors it is useful for several different target-binding ligands to be available. Effective targeted transfection requires not only good targeting but also effective transfer of the vector nucleic acid to the nucleus of the target cell. Even if a ligand is effective in targeting and binding to a target cell, effective gene transfection does not always occur. The reasons for that are, at present, not clear. Accordingly, there remains a degree of unpredictability regarding whether a ligand that binds effectively to a target cell will also bring about effective transfection. It is therefore desirable to have available a "pool" of ligands for any particular cell surface receptor from which an effective transfection ligand may be selected. Such selection may take place by means of a gene transfer assay using, for example, a reporter gene, or by any other suitable means.

SUMMARY OF THE INVENTION

The invention is based on the identification of peptides comprising specific amino acid motifs, which peptides bind to human immature dendritic cells. The identified peptide motifs mediate binding to human immature dendritic cells and

also to other types of cells.

The present invention provides a peptide having, consisting of or comprising an amino acid sequence selected from

a) $PX^1X^2X^3T$ [SEQ.ID.NO.:1];
b) PSX^4S [SEQ.ID.NO.:2];
c) $QX^5X^6X^7Q$ [SEQ.ID.NO.:3];
d) SX^8S [SEQ.ID.NO.:4],

in which X^1, X^2 and X^3, which may be the same or different, each represents an amino acid residue;
X^4 represents an amino acid residue;
X^5 and X^7, which may be the same or different, each represents an amino acid residue, and X^6 represents an amino acid residue having an amide side chain, for example, N or Q.
X^8 represents an amino acid residue having an aliphatic side chain, for example, L or I.

The conventional single letter system of abbreviation is used herein to denote amino acids.

The invention provides the use of a peptide of the invention to target an entity may be a nucleic acid or another molecule, for example, a therapeutically or pharmaceutically active molecule, or a molecule comprising a detectable label.

The present invention also provides a peptide derivative of the formula A-B-C in which
A denotes a peptide of the present invention,
B denotes a chemical bond or a spacer element, and
C denotes a polycationic nucleic acid binding component.

The present invention further provides a transfection mixture that comprises

(i) lipid component,
(ii) a polycationic nucleic acid binding component, and
(iii) a peptide of the invention.

The present invention further provides a non-viral transfection complex that comprises
(i) lipid component,
(ii) a polycationic nucleic acid binding component, and
(iii) a peptide of the invention, and
(iv) a nucleic acid.

In a transfection mixture or transfection complex of the invention components (ii) and (iii) are preferably in the form of a peptide derivative of the invention.

The invention also provides a viral vector, which vector comprises a peptide of the invention.

In one embodiment, the viral vector is an adenovirus retargeted from its native CAR receptor by incorporation of a peptide of the invention in the adenoviral vector, for example, in the HI region of the fibre protein in the capsid, for example, of adenovirus type 5.

The invention also provides processes for the production of a transfection mixture, a transfection complex, and a viral vector of the invention.

The invention further provides a pharmaceutical composition which comprises a transfection mixture, transfection complex or viral vector of the invention in admixture or conjunction with a pharmaceutically suitable carrier.

The invention further provides a method for the treatment or prophylaxis of a condition caused in a human or in a non-

human animal by a defect and/or a deficiency in a gene which comprises administering a transfection complex or a viral vector of the invention the human or to the non-human animal.

The term "a defect and/or a deficiency in a gene" as used herein denotes not only a defect or deficiency in the coding region of a gene, but a defect or deficiency in a control element for the gene, for example, a control element in trans or in cis, or a defect or deficiency in any other element that is involved in the transcription or translation of of the gene, whether directly or indirectly.

The invention further provides a method for therapeutic or prophylactic immunisation of a human or of a non-human animal, which comprises administering a transfection complex or a viral vector of the invention comprising an anti-sense nucleic acid to the human or to the non-human animal.

The invention also provides a method of anti-sense therapy, which comprises administering a transfection complex or a viral vector of the invention to a human or to a non-human animal.

The invention further provides a transfection complex or a viral vector of the invention for use as a medicament or a vaccine.

The invention also provides the use of a transfection complex or a viral vector of the invention for the manufacture of a medicament for the prophylaxis of a condition caused in a human or a non-human animal by a defect and/or a deficiency in a gene, or for therapeutic or prophylactic immunisation, or for anti-sense therapy.

The invention additionally provides a kit that comprises

(i) nucleic acid,

(ii) a lipid component,

(iii) a polycationic nucleic acid-binding component, and

(iv) a peptide of the invention;

a kit that comprises

(i) nucleic acid,

(iii) a polycationic nucleic acid-binding component, and

(iv) a peptide of the invention; and

a kit that comprises

(ii) a lipid component,

(iii) a polycationic nucleic acid-binding component, and

(iv) a peptide of the invention.

In a kit of the invention, components (iii) and (iv) are preferably in the form of a peptide derivative of the invention, as described above.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the results of titration of phage clone binding to human and mouse dendritic cells, with binding to plastic as a control. The human monocyte-derived immature dendritic cells were obtained from donors JD, S and LA. Numbers of phage are shown as as plaque forming units (pfu). Subsequent sequencing of the bound phage identified peptides APSNSTA, QLLTGAS, TARDYRL, FQSQYQK, PLMPSLS, FPRAPHH, MASISMK, DWWHTSA, SHVKLNS and SPALKTV and also denotes a phage with no inserted peptide.

Figure 2 shows binding of phage clones to human monocyte-derived immature dendritic cells from donors SB, U, SH, KG and AM, as measured by fluorescence activate cell sorting (FACs). The percentage cells positive for FITC was measured

by FACs analysis. Subsequent sequencing of the bound phage identified peptides APSNSTA, FQSQYQK, DWWHTSA, SHVKLNS, SPALKTV and SQKNPQM.

Figure 3 shows phage clone binding to human monocyte-derived immature dendritic cells from donors KG and SB as measured by FACs. Subsequent sequencing of the bound phage identified peptides QLLTGAS, TARDYRL, PLMPSLS, FPRAPHH, MASISMK, STPPNTT.

Figure 4 shows transfection of human monocyte-derived immature dendritic cells using phage-derived targeting peptides in a transfection vector. Transfection of human monocyte-derived dendritic cells with phage derived peptide A derivative ($[K]_{16}$-GACSHVKLNSCG), peptide B derivative ($[K]_{16}$-GACAPSNSTACG), peptide 6 derivative $[K]_{16}$-GACRREEWACG) or the scrambled control peptide 6J ($[K]_{16}$-GACATRWARECG). Peptide A and B derivatives are used in a transfection complex in a ratio to phage DNA of 1.5:1 (A1.5, B1.5) 3:1 (A3), B3, and 7:1 (A7, B7). Controls include cells with no transfection complexes added (OptiMEM only), and also peptide 6 derivative and peptide 6J, its scrambled control both at 3:1 ratio of peptide :DNA. Each result is the percentage GFP positive cells from 3 pooled transfection reactions.

Figure 5 shows transfection of HMEC-1 cell line with phage-derived peptide A and B derivatives (see the legend to Figure 4 above). The results are given in RLU/mg, RLU denoting relative light unit. Transfection of cells with peptide A and B derivatives and the control peptide 6 derivative and its scrambled control peptide 6J was carried out with a range of peptide:DNA charge ratios including 3:1, 5:1 and 7:1 (A3, A5, A7, B3, B5, and B7, 6 3, 6 5 and 6J 5). Controls include cells with no transfection complexes added (OptiMEM only),

peptide 6 derivative, an integrin binding peptide and 6J, its scrambled control. Each result is the percentage GFP positive cells from 3 pooled transfection reactions. Each result is the mean of 6 values and error bars represent the standard deviation about the mean.

Figure 6 shows transfection of HAEo cell line with phage-derived synthesised peptides and controls as described for Figure 5.

Figure 7 shows transfection of N2a cell line with phage-derived synthesized peptides and controls as described for Figure 5.

Figure 8 shows transfection of human monocyte-derived immature dendritic cells with adenovirus retargeted by incorporating peptide A (columns A) or peptide B (columns B) in the HI region of the fibre protein in the capsid, with a KO1 fibre protein in the capsid (columns KO1), and with wild-type fibre protein in the capsid (columns WT). The darker shaded columns show % transduction, the lighter shaded columns show % cell death.

DETAILED DESCRIPTION OF THE INVENTION

The invention is based on the identification of peptides comprising specific amino acid motifs, which peptides that bind to human immature dendritic cells. The identified peptide motifs mediate binding to human immature monocyte - derived dendritic cells and also to other types of cells, including human primary macrophages, N2a cells (mouse neuroblastoma cell line), HAEo (human airway epithelial cell line) cells; HepG2 (human hepatocyte cell line) and primary mouse cells including bone-marrow derived dendritic cells and Sca1+ve mouse stem cells.

The present invention provides a peptide having, consisting of or comprising an amino acid sequence selected from

a) $PX^1X^2X^3T$ [SEQ.ID.NO.:1];
b) PSX^4S [SEQ.ID.NO.:2];
c) $QX^5X^6X^7Q$ [SEQ.ID.NO.:3];
d) SX^8S [SEQ.ID.NO.:4],

in which X^1, X^2 and X^3, which may be the same or different, each represents an amino acid residue;
X^4 represents an amino acid;
X^5 and X^7, which may be the same or different, each represents an amino acid residue, and X^6 represents an amino acid residue having an amide side chain, for example, N or Q;
X^8 represents an amino acid residue having an aliphatic side chain, for example, L or I.

The conventional single letter system of abbreviation is used herein to denote amino acids. According to that system A denotes (=) alanine, R = arginine, N = asparagine, D = aspartic acid, C = cysteine and cystine, G = glycine, E = glutamic acid, Q = glutamine, H = histidine, I = isoleucine, L = leucine, K = lysine, M = methionine, F = phenylalanine, P = proline, S = serine, T = threonine, W = tryptophan, Y = tyrosine, and V = valine.

In a peptide $PX^1X^2X^3T$ [SEQ.ID.NO.:1] X^1 may be, for example, S, A or P. X^2 may be, for example, N or L. X^3 may be, for example, S, K or T.

A peptide of $PX^1X^2X^3T$ in which X^2 represents L is a peptide PX^1LX^3T [SEQ.ID.NO.:5]. X^1 and X^3, which are the same or different, may be as described above. For example, X^1 may represent S, A or P, for example, A. X^3 may, for example, represent S, K or Y, for example, K. An example of a peptide

PX^1LX^3T is peptide PALKT [SEQ.ID.NO.:6].

In a peptide PX^1LX^3T [SEQ.ID.NO.:1], X^2 may represent N, which peptide is PX^1NX^3T [SEQ.ID.NO.:7]. X^1 and X^3 may be the same or different. X^1 is, for example, S or P, for example, S. X^3 is, for example, S or T, for example, S. Both X^1 and X^3 may be S. Examples of a peptide PX^1NX^3T are peptide PSNST [SEQ.ID.NO.:8], and PPNTT [SEQ.ID. NO. 9].

A peptide $PX^1X^2X^3T$ [SEQ.ID.NO.:1] may have, independently, one or more additional residues at the N-terminus and/or at the C-terminus. For example,

a peptide $PX^1X^2X^3T$, for example, any of the peptides of SEQ.ID.NO.:1 described above, may also comprise an additional residue. for example, an A or V residue at the C-terminus. Such a peptide has the sequence $PX^1X^2X^3TX^9$ [SEQ.ID.NO.:10] in which X^9 represents an amino acid residue, for example, A or V. Examples of such peptides are $PX^1LX^3TX^9$ [SEQ.ID.NO.:11] and $PX^1NX^3TX^9$ [SEQ.ID.NO.:12].

Independently, a peptide $PX^1X^2X^3T$ may have an additional residue at the N-terminus, which peptide has the sequence $X^{10}PX^1X^2X^3T$ [SEQ. ID. No.:13] in which X^{10} represents an amino acid residue, for example, an A, S or T residue.

When an additional residue is present at both the N-terminus and the C-terminus the peptide has the sequence $X^{10}PX^1X^2X^3TX^9$ [SEQ.ID.NO.: 14] in a peptide of $X^{10}PX^1X^2X^3TX^9$, X^1, X^2, X^3, X^9 and X^{10} may have the preferred meanings given above.

Examples of peptides of SEQ.ID.NO.:1 having additional residues include APSNSTA [SEQ.ID.NO.:15], SPALKTV [SEQ.ID.NO.:16] and STPPNTT [SEQ.ID.NO.:17]. Variants of such peptides have the N-terminal and/or C-terminal residue

omitted.

In a peptide PSX4S [SEQ.ID.NO.:2], X^4 may be, for example, N or L. Examples of peptides of PSX^4S include PSNS [SEQ.ID.NO.:18] and PSLS [SEQ.ID.NO.:19].

A peptide of PSX^4S may have, independently, one or more amino acid residues at the N-terminus and/or the C-terminus, for example, an A or L residue at the N-terminus, giving a peptide $X^{11}PSX^4S$ [SEQ.ID.NO.:20] in which X^{11} represents A or L. In such a peptide X^4 may be N or L. Examples of such peptides include APSNS [SEQ.ID.NO.:21] and LPSLS[SEQ.ID.NO.:22]. If desired, one or more further residues may be present at the N-terminus, for example, as in peptides, MLPSLS [SEQ.ID.NO.:23] and PMLPSLS [SEQ.ID.NO.:24].

In a peptide $QX^5X^6X^7Q$ [SEQ.ID.NO.:3], X^6 may be an N or Q residue. X^3 may be, for example, K or S. X^5 may be, for example, P or Y.

A peptide $QX^5X^6X^7Q$ may have, independently, one or more amino acid residues at the N-terminus and/or the C-terminus. A peptide of $QX^5X^6X^7Q$ may have, for example, an N-terminal S or F residue and may have, independently, an M or K residue at the C-terminus. Such peptides include, for example, SQKNPQM [SEQ.ID.NO.25:] and FQSQYQK [SEQ.ID.NO.:26] and variants in which the N- and/or C-terminal residue is omitted.

A further peptide of the invention has the motif $SX^{10}S$ [SEQ.ID.NO.:4], in which X^{10} is L or I. A peptide $SX^{10}S$ may have, independently, one or more amino acid residues at the N-terminus and/or the C-terminus. Examples of such peptides include MASISMK [SEQ.ID.NO.:27], and derivatives thereof in which any one or more of the N-terminal and/or C-terminal residues are omitted.

A peptide of the invention may be up to 30 amino acids in length, or may be longer. A peptide of the invention generally has at least about 5 amino acids but may have fewer, for example, as in the case of peptides of SEQ.ID.NOs. 2 and 3]. Generally, a peptide of the invention has any number of amino acids from about 6 to about 30 inclusive. The peptide may have 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26 ,27, 28, 29 or 30 amino acids. Generally, a peptide of the invention may have 25 or fewer amino acids, for example, 20 or fewer, for example 15 or fewer. For example, a peptide of the invention may have 12 amino acids or fewer, for example, 10 amino acids or fewer. Generally, it is preferred for a peptide of the invention to have 5 or more amino acids. For example, a peptide of the invention may have 6 or more amino acids, for example 7 or more amino acids. In the case of a peptide comprising SEQ.ID.NO.:2, the minimum size is 4 amino acids; in the case of a peptide comprising SEQ.ID.NO.:1 the minimum size is 5 amino acids; in the case of a peptide comprising.SEQ. ID. NO. 3 the minimum size is 5 amino acids.

A peptide of the invention may comprise a cyclic region. For example, a motif of the invention is flanked by two or more cysteine residues that are capable of forming one or more disulphide bond(s).

In certain cases it may be desirable that the peptide is larger than described above. A peptide of the invention may be part of a recombinant polypeptide or part of a fusion protein, for example fused to a amino acid sequence that has a desired function, for example, a sequence suitable for use in affinity chromatography. A further example of a fusion protein comprises a peptide of the invention and a viral capsid protein or a region thereof for targeted delivery, or

a protein that facilitates peptide display for targeting purposes.

A further fusion protein comprises a peptide of the invention and an antibody against a viral capsid protein or subunits thereof. The antibody component may be of any antibody class, may be an appropriate antigen-binding domain or domains, and may be or be derived from a chimeric or humanised antibody. Such a fusion protein, which may be used in retargeting a viral vector, is part of the present invention.

The peptides of the invention bind selectively to dendritic cells, in particular to human dendritic cells, for example to immature human dendritic cells and mouse dendritic cells. The peptides of the invention may therefore be used to target desired entities to such cells. For example, a peptide of the invention may be used to target a nucleic acid or an antigen to dendritic cells. A peptide of the invention may be used to target a pharmaceutically active substance to dendritic cells.

It is not yet known to which receptor(s) on dendritic cells the peptides of the invention bind. However, we have found that the peptides of the present invention bind to other cells and other types of cells, including including human primary macrophages, N2a (mouse neuroblastoma cell line) cells, HAEo (human airway epithelial cell line) cells, HepG2 (human hepatocyte cell line) cells and primary mouse cells including bone-marrow derived dendritic cells and Sca1+ve mouse stem cells. A peptide of the invention may be used to target entities, for example, nucleic acids, antigens and pharmaceutically active substances, to such cells.

Identification of peptides of the invention and their binding

to different cell types may be determined readily, for example, by a phage peptide clone screening assay using either whole cell flow cytometry, for example, FACS, or titration of phage, or by transduction of cells with retargeted adenovirus bearing the peptide of interest. Such assays are described below and in detail in the following Examples see, for example, Example 1, Material & Methods.

A peptide of the present invention, for example, a dendritic cell-binding component, may be identified by selection from a peptide library of oligomeric peptides, for example, a library of random peptide oligomers, generally of the same length. While in principle the oligomeric peptides may be of any length, a peptide that is too long may present difficulties of synthesis and may be immunogenic *in vivo*, while a peptide that is too short may not have any binding domain. Examples of targeting motifs that are generally suitable are those having from about four to about 30 amino acid residues, see above.

In studies that are described in detail in the Examples below, random 7-mers (peptides having seven amino acid residues) and random 12-mers (peptides having twelve amino acid residues) displayed on filamentous phage particles were used. In those studies results obtained using the random 7-mer library were better than those using the random 12-mer peptide library. However, the reasons for the difference in performance of the seven and twelve amino acid libraries are not known at present, and such differences may not be found in future work. It is possible that the larger amino acid insert in the phage coat protein reduces the viability of the phage and/or that the additional protein synthesis requirement places too great a burden on the E.coli bacteria. Alternatively, or in addition, impurities in or defects of the 12-mer library may have adversely affected the outcome of

the experiments with that library. It appears at present that smaller peptides, for example heptameric peptides may be preferred. Accordingly, it may be preferable that a peptide of the invention has from about 4 to 11 amino acids, for example, from about 4 to 10 amino acids, for example, from about five to ten, for example, 6, 7, 8 or 9 amino acids. However, it may alternatively be preferable to used larger peptides.

The 7-mer library used was a C7C library i.e. random 7-mer peptides flanked by cysteine residues, obtained from New England Biolabs Inc. The 12-mer library used was also obtained from New England Biolabs Inc.

As indicated above, the dendritic cell binding peptides of the invention were identified by selection from a phage display library comprising random peptide sequences seven residues in length flanked by cysteine residues to allow cyclisation. Such selection procedures are generally known. According to such procedures, suspensions of phage are incubated with target cells. Unbound phage are then washed away and, subsequently, bound phage are extracted either by washing the remaining cells with a low pH buffer or by lysing the cells. *E. coli* are then infected with released phage and a preparation of first round phage is obtained. The cycle is performed repeatedly, for example, three times and, in order to enrich for targeting phage, the stringency conditions may be increased in the later rounds of selection, for example by increasing the number of wash steps, introducing a low pH wash prior to elution and preselecting with wells coated with medium blocker.

Following selection by successive rounds of phage amplification, we found that phage with high affinity for dendritic cells may be selected further by whole cell flow

cytometry and phage titration assays.

The amino acid sequences of clones obtained from cell lysis eluted C7C phage in a first experiment are shown in Table 1.

TABLE 1

Sequence	Clone frequency (%)	SEQ.ID
APSNSTA	21	15
DWWHTSA	20	28
SHVKLNS	12	29
SQKNPQM	7	25
QLLTGAS	6	30
SPALKTV	6	16
FQSQYQK	6	26
TARDYRL	5	31
FPRAPHH	5	32
STPPNTT	4	17
PMLPSLS	1	24
SEWLSAL	1	33
IGGIRRH	1	34
YTMEFNR	1	35
MASISMK	1	27
PAAYKAH	1	36

Each of the peptides listed above is part of the present invention, as are longer and shorter derivatives thereof, for

example, as described above.

Analysis of the 16 binding sequences from the phage clones identified five minimal motifs, namely PXNT/ST, PXXXTA/V, A/LPSXS, SL/IS AND QXN/QXQ, which were considered to potentially to play an important role in binding to receptors on dendritic cells. Of the clones sequenced, 46% contained one or more of the five motifs, with the most frequent clone, APSNSTA, showing a degree of homology to three other peptide sequences, SPALKTV, STPPNTT and PMLPSLS.

Phage were recovered and titred from each round of phage clone binding to immature dendritic cells. To summarise the procedure, $2x10^{11}$blocked phage were added to 5×10^4 blocked monocyte-derived immature dendritic cells for 1 hour on ice before washing cells three times with PBS-0.05% Tween 20, eluting phage with TBS pH5.5, and lysing cells to harvest the phage remaining bound. The numbers of phage harvested by cell lysis were calculated as plaque forming units (pfu). Figure 1 shows results of some of the titrations. Sequencing of the bound phage shown in Figure 1 identified the peptides as APSNSTA, QLLTGAS, TARDYRL, FQSQYQK, PLMPSLS, FPRAPHH, MASISMK, DWWHTSA, SHVKLNS, and SPALKTV.

Sequencing of 81 phage clones from the cell-associated fraction from the third round of titration of phage clone binding to immature dendritic cells identified 16 different sequences, see Table 2.

TABLE 2 Phage sequences from third round of titration of phage clone binding to dendritic cells

Sequence	Number of clones	Percentage of clones
APSNSTA	17	21
DWWHTSA	16	20
SHVKLNS	10	12
SQKNPQM	6	7
QLLTGAS	5	6
SPALKTV	5	6
FQSQYQK	5	6
TARDYRL	4	5
FPRAPHH	4	5
STPPNTT	3	4
PMLPSLS	1	1
SEWLSAL	1	1
IGGIRRH	1	1
YTMEFNR	1	1
MASISMK	1	1
PAAYKAH	1	1

The three most frequent phage clones are present at 21% (APSNSTA), 20% (DWWHTSA) and 12% (SHVKLNS), with the remainder present at 7% and below. Analysis of the 16 binding sequences from the phage clones identified five minimal motifs, namely, $PXN^{T}/_{S}T$, $PXXXT^{A}/_{V}$, $^{A}/_{L}PSXS$, $S^{L}/_{I}S$, and $QX^{N}/_{Q}XQ$, see Table 2B, which motifs may play an important role in binding to receptors on dendritic cells. Of all the clones sequenced, 46% contained one or more motifs, with the most frequent clone, APSNSTA, showing a degree of homology to three other peptide sequences, see Table 3.

TABLE 3 Conserved amino acid motifs in peptide sequences

Peptide Homology	Motif	% clones containing motif
A***P***SNS***T***A S***P***ALK***T******V***	PXXX$T^{A}/_{V}$	27
ST***P******P******N******T******T*** A***P******S******N******S******T***A	PXNXT	25
A***P******S***N***S***TA PM***L******P******S***L***S***	$^{A}/_{L}$PSXS	22
S***Q***K***N***P***Q***M F***Q***S***Q***Y***Q***K	$QX^{N}/_{Q}XQ$	13
PMLP***S******L******S*** MA***S******I******S***MK	$S^{L}/_{I}S$	2

Identical amino acids are shown in bold and italic
Similar amino acids are shown in italic

Titrations of phage clone binding to dendritic cells in most cases showed that the clones having peptide inserts bind to a greater extent to the cells than do phage that have no insert in the cells. Two clones, FPRAPHH and MASISMK bound in highest numbers in all titrations, including the titration of phage binding to mouse dendritic cells. The numbers of phage binding to plastic were low for all clones tested, suggesting that phage binding demonstrated by high titres in these experiments is due to binding to cells and not background non-specific binding to the wells or blocking molecules.

FACs analysis of phage binding to dendritic cells from five different dendritic cell donors (SB, U, SH, KG and AM) with six of the most frequent clones namely APSNSTA, FQSQYQK, DWWHTSA, SHVKLNS, SPALKTV, and SQKNPQM showed that all clones except for one, SPALKTV, were detected binding to a higher percentage of cells than a phage clone bearing no insert, see Figure 2.

The pattern of binding identified the three clones that bind to dendritic cells in highest amounts as those containing the peptides APSNSTA,DWWHTSA and SHVKLNS, which clones were also the three most frequently isolated from the selection. For the second set of six clones tested, namely those containing the peptides QLLTGAS, TARDYRL, PMLPSLS, FPRAPHH, MASISMK, and STPPNTT, all clones showed a higher percentage of cells positive for bound phage than the controls with no insert. QLLTGAS binds to marginally more cells than the others, see Figure 3, in which the dendritic cell donors are KG and SB.

Of the 16 phage sequences two, namely APSNSTA and SHVKLNS, were chosen for synthesis on the basis that they were amongst the most frequent clones and also were two of the top binders in the FACs assay of phage clone binding. SHVKLNS is called "peptide A" and APSNSTA is called "peptide B".

From the Tables it may be seen that motifs were present in several of the clones. This strongly suggests that those motifs are important for dendritic cell binding. It is at present not known to which dendritic cell receptor(s) the sequences bind. The various motifs may target the same receptor or they may target different receptors on dendritic cells. The receptor(s) also occur on other cells, see below.

Good binding indicates a high affinity interaction and/or the binding of a cell surface receptor molecule present in high numbers on the cell surface.

Peptides A and B were synthesized in a constrained form with a DNA-binding $[K]_{16}$ domain, a GAC (inter and a C-terminal CG groyp. The peptides were tested for their ability to transfect immature dendritic cells in a transfection complex with DNA and lipid (a lipopolyplex vector). The results are shown in Figure 4.

Transfection efficiency, as measured by the percentage of cells positive for the reporter gene EGFP determined by FACs, was increased by the use of the phage-derived peptide A (SHVKLNS) to a level approximately one and a half times that of the positive control, peptide 6, which is an integrin binding peptide RRETAWA and the negative control, peptide 6J, (a scrambled version of peptide 6). Peptide B (APSNSTA) produced transfection levels equal to those of peptide 6. The percentage of cells transfected did not reach 5%, possibly due to the toxic effect of the transfection procedure on the dendritic cells.

Although the peptide sequences of the invention were identified using dendritic cells, their utility is not limited to use with dendritic cells. The receptors to which the peptides bind may be expressed in other cell types. Cell types with which peptides of the invention may be used may be identified by any suitable screening procedure.

For example, peptides A and B in transfection vectors were tested for their ability to transfect other cell lines. The transfection efficiency was measured by luciferase activity per mg of protein present. In all cell lines tested, namely HMEC-1, HAEo- and N2a cells, at least one of the peptides A and B produced transfection efficiencies equal to or above those seen with peptide 6, the integrin targeting peptide.

In HMEC-1 cells, whilst peptide A produces a transfection efficiency approximately equal to that seen with peptide 6, peptide B can increase efficiency by one and a half times that of peptide 6, see Figure 5.

In HAEo- cells, peptide A produced the highest efficiency of transfection, approximately double that seen with peptide 6,

whilst efficiency using peptide B was one and a half times that using peptide 6, see Figure 6.

In N2a cells, only peptide A produced transfection efficiencies equal to those seen with peptide 6, with peptide B producing efficiencies of less than half that value, see Figure 7.

Furthermore, as described herein, the peptides of the invention may be used to retarget viral vectors to cells other than their normal targets.

Gene transfer of EGFP-reporter gene-bearing adenoviral constructs retargeted to immature dendritic cells from two different donors at 100,000 viral particles/cell in complete medium was measured by FACs. In both donors, retargeting the adenovirus with either peptide A or peptide B produced a transduction efficiency of between 64 and 79%, both being of similar efficiencies, and both transducing a significantly higher percentage of cells compared with adenovirus with a wild type fibre protein in the capsid, (between 20 and 46% positive), a KO1 fibre protein (between 0.7 and 1.4 %), and a fibre protein bearing an irrelevant peptide (between 12 and 43 %), see Table 4. No significant toxicity was seen in any transduction, with cell death being measured at between 5 and 15%, see Figure 8.

Using the same viral vectors, transduction of human monocyte-derived primary macrophages at 10,000 viral particles/cell in 2.5% serum also demonstrated that incorporating either peptide A (67.6% cells transduced) or peptide B (34.6% of cells transduced) into the viral coat significantly increased the efficiency of transduction above that seen with virus bearing a wild type fibre protein (13.3%) or KO1 fibre protein (9.2% cells transduced), see Table 4 and Figure 8.

TABLE 4 Transduction of human monocyte-derived primary macrophages

	Wild type	KO1	Irrelevant	A	B
Primary human macrophages	13.3	9.2	N/D	67.6	34.6

In all other cell types, and using the same viral vectors, when virus was added at 10,000 particles per cell in OptiMEM, virus bearing peptide A or peptide B resulted in significantly higher transduction efficiency than virus bearing wild type fibre protein, KO1 fibre protein or fibre protein bearing an irrelevant peptide, see Table 5.

TABLE 5. Transduction of other cell types

Cell type	Virus				
	Wild type	KO1	Irrelevant	A	B
N2a	24.3	1.4	1.3	63.5	53.7
HAEo-	45.8	2.0	3.2	82.9	79.0
CdcHMEC-1	73.3	2.7	25.1	95.7	94.2
HepG2	63.8	1.7	9.7	88.5	79.7

In N2a cells, virus bearing peptide A produced 63.5% tranduced cells, peptide B 53.7%, whilst wild type produced 24.3, KO1 1.4% and irrelevant peptide 1.3%.

In HAEo- cells, peptide A produced 82.9% transduced cells, peptide B 79%, whereas wild type fibre bearing virus

transduced 45.8%, KO1 virus 2% and virus bearing an irrelevant peptide 3.2%.

The same patterns of transduction efficiencies were seen with HMEC cells (peptide A 95.7%, peptide B 94.2%, wild type 73.3, KO1 2.7% and irrelevant 25.1%) and HepG2 cells (peptide A 88.5%, peptide B 79.7%, wild type 63.8%, KO1 1.7% and irrelevant 9.7%).

In so far as any motif or any peptide of the invention occurs in a known naturally-occurring protein, the peptides of invention do not include such a naturally-occurring full-length protein *per se*. Generally, the peptides of the invention are 100 or fewer amino acids in length; for example, 50 or fewer amino acids in length. Typically, they are of sizes described above.

The present invention also provides a peptide derivative of formula A-B-C wherein

A is a polycationic nucleic acid-binding component,
B is a chemical bond or a spacer element, and
C is a peptide of the present invention.

The polycationic nucleic acid-binding component A is any polycation that is capable of binding to DNA or RNA. A poly-cation may be polycationic itself or it may have any number of cationic monomers provided the ability to bind to DNA or RNA is retained. For example, from 3 to 100 cationic monomers may be present, for example, from 10 to 20, for example from 14 to 18, for example, about 16.

An example of a nucleic acid-binding polycationic molecule is an oligopeptide comprising one or more cationic amino acids. Such a oligopeptide may, for example, be an oligo-lysine molecule having, for example, from 3 to 35, for example, 5 to

25 lysine residues, for example, having from 10 to 20 lysine residues, for example, from 14 to 18 lysine residues, for example, 16 lysine residues, an oligo-histidine molecule or an oligo-arginine molecule having, for example, from 3 to 35, for example, from 5 to 25, for example, from 10 to 20, for example, from 14 to 18, for example, 16 histidine or arginine residues, respectively, or a combined oligomer comprising any combination of histidine, arginine and lysine residues and having, for example, a total of from 3 to 35, for example, from 5 to 25 residues, preferably for example, from 10 to 20 residues, for example, from 14 to 18 residues, for example 16 residues.

An oligolysine is particularly preferred, for example, having from 3 to 35, for example, from 2 to 25, for example, form 10 to 20 lysine residues, for example, from 13 to 19, for example, from 14 to 18, for example, from 15 to 17 residues, for example, 16 residues i.e. $[K]_{16}$, "K" denoting lysine.

Further examples of polycationic components include dendrimers and polyethylenimine. Polyethylenimine (PEI) is a non-toxic, cross-linked cationic polymer with gene delivery potential (*Proc. Natl. Acad. Sci.*, 1995, **92**, 7297-7301). Polyethylenimine is obtainable from Fluka (800kDa) or from Sigma (50kDa) or alternatively pre-diluted for transfection purposes from PolyPlus-tranfection (Illkirch, France). Typically, PEI is most efficient when used in a 9 fold excess over DNA, the excess ratio being calculated as PEI nitrogen : DNA phosphate, and at pH 5 to 8. Such parameters may optimised in a manner familiar to the person skilled in the art.

The polycationic nucleic acid-binding component may be linked or otherwise attached to the peptide of the invention to form a peptide derivative of the invention A-B-C in which C

denotes a peptide of the present invention, B denotes a chemical bond or a spacer element, and A denotes a polycationic nucleic acid binding component. The polycationic component may be linked at any appropriate position of the peptide. A polycationic nucleic acid-binding component may, for example, be chemically bonded directly to a peptide of the invention, in which case the component B represents a chemical bond. For example, a peptide of the invention may be linked by a peptide bond, for example, in the case of an oligolysine polycationic nucleic acid-binding component. An example of a peptide derivative of the invention is an oligolysine, for example, $[K]_{16}$, linked via a peptide bond to a peptide of the invention, for example, a peptide as described above. A further example of a peptide derivative of the invention is a polyethylenimine linked via a covalent link to a peptide of the invention, for example, a peptide as described above. Such a covalent link may be, for example, a disulphide bridge or a succinimidyl bridge, using methods known in the art see for example, *Gene Therapy*, 1999, **6**, 138-145).

In another embodiment, a peptide of the invention may be attached to a polycationic nucleic acid binding component via a spacer to form a peptide derivative of the invention.

A spacer element is generally a peptide, that is to say, it comprises amino acid residues. The amino acids may be naturally occurring or non-naturally occurring. They may have L- or D-configuration. A spacer may have two or more amino acids. It may, for example, comprise three or more amino acids, for example, four or more, for example, five or more, for example, up to ten amino acids or more. The amino acids may be the same or different, but the use of multiple lysine residues (or other cationic amino acids suitable for use in the polycationic nucleic acid-binding component of a

vector complex) should generally be avoided in the spacer as oligo-lysine sequences have activity as a polycationic nucleic acid-binding component.

The spacer may be, for example, the dipeptide glycine-glycine (GG) or glycine-alanine (GA). Generally it is preferable that the spacer is longer and/or more hydrophobic than the dipeptide spacers GG and GA.

The spacer may be more hydrophobic than the dipeptides GG and GA. For example, amino acids that are more hydrophobic than glycine and alanine may be used. Examples of hydrophobic amino acids are well known and include Є-amino hexanoic acid.

A spacer may be either longer or more hydrophobic than the dipeptides GG and GA, or it may be both longer and more hydrophobic. An example of the latter type of spacer is XSXGA, wherein S = serine, G = glycine, A = alanine and X = Є-amino hexanoic acid. This spacer is highly hydrophobic.

A combined peptide/polycationic nucleic acid binding component i.e. a peptide derivative of the invention may be referred to below as component "I".

The present invention further provides a transfection mixture that comprises
(i) lipid component,
(ii) a polycationic nucleic acid binding component, and
(iii) a peptide of the invention.

The present invention also provides a non-viral transfection transfection complex that comprises
(i) lipid component,
(ii) a polycationic nucleic acid binding component, and
(iii) a peptide of the invention, and

(iv) a nucleic acid.

In a transfection mixture or transfection complex of the invention, components (ii) and (iii) are preferably in the form of a peptide derivative of the invention, for example, as described above.

The lipid component of a transfection mixture or transfection complex of the invention may be or may form a cationic liposome.

The lipid component may be or may comprise one or more lipids selected from cationic lipids and lipids having membrane destabilising or fusogenic properties, especially a combination of a cationic lipid and a lipid that has membrane destabilising properties.

A preferred lipid component ("L") is or comprises the neutral lipid dioleyl phosphatidylethanolamine, referred to herein as "DOPE". DOPE has membrane destabilising properties sometimes referred to as "fusogenic" properties (Farhood et al. 1995). Other lipids, for example, neutral lipids, having membrane destabilising properties, especially membrane destabilising properties like those of DOPE may be used instead of or as well as DOPE.

Other phospholipids having at least one long chain alkyl group, for example, di(long alkyl chain)phospholipids may be used. The phospholipid may comprise a phosphatidyl group, for example, a phosphatidylalkanolamine group, for example, a phosphatidyl-ethanolamine group.

A further preferred lipid component is or comprises the cationic lipid N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethyl-ammonium chloride, referred to herein as "DOTMA". DOTMA has

cationic properties. Other cationic lipids may be used in addition to or as an alternative to DOTMA, in particular cationic lipids having similar properties to those of DOTMA. Such lipids are, for example, quaternary ammonium salts substituted by three short chain alkyl groups, and one long chain alkyl group. The short chain alkyl groups may be the same or different, and may be selected from methyl and ethyl groups. At least one and up to three of the short chain alkyl group may be a methyl group. The long alkyl chain group may have a straight or branched chain, for example, a di(long chain alkyl)alkyl group.

Another preferred lipid component is or comprises the lipid 2,3-dioleyloxy-N-[2-(spermidinecarboxamido)ethyl]-N,N-dimethyl-1-propanaminiumtrifluoridoacetate, referred to herein as "DOSPA". Analogous lipids may be used in addition to or as an alternative to DOSPA, in particular lipids having similar properties to those of DOSPA. Such lipids have, for example, different short chain alkyl groups from those in DOSPA.

A preferred lipid component comprises DOPE and one or more other lipid components, for example, as described above. Especially preferred is a lipid component that comprises a mixture of DOPE and DOTMA. Such mixtures form cationic liposomes. An equimolar mixture of DOPE and DOTMA is found to be particularly effective. Such a mixture is known generically as "lipofectin" and is available commercially under the name "Lipofectin". The term "lipofectin" is used herein generically to denote an equimolar mixture of DOPE and DOTMA. Other mixtures of lipids that are cationic liposomes having similar properties to lipofectin may be used. Lipofectin is particularly useful as it is effective in all cell types tested.

A further preferred lipid component comprises a mixture of DOPE and DOSPA. Such mixtures also form cationic liposomes. A mixture of DOPE and DOSPA in a ratio by weight 3:1 DOSPA:DOPE is particularly effective. Such a mixture, in membrane filtered water, is available commercially under the name "Lipofectamine". Mixtures comprising DOPE, DOTMA and DOSPA may be used, for example, mixtures of lipofectin and lipofectamine.

Other cationic lipids are available commercially, for example, DOTAP (Boehringer-Mannheim) and lipids in the Tfx range (Promega). DOTAP is N-[1-(2,3-diolyloxy)propyl]-N,N,N-trimethylammonium methylsulphate. The Tfx reagents are mixtures of a synthetic cationic lipid [N,N,N',N'-tetramethyl-N,N'-bis(2-hydroxyethyl)-2,3-di(oleoyloxy)-1,4-butanediammonium iodide and DOPE. All the reagents contain the same amount of the cationic lipid component but contain different molar amounts of the fusogneic lipid, DOPE.

However, lipofectin and lipofectamine appear to be markedly more effective for promoting transfection of cells with a nucleic acid than are DOTPA and Tfx agents.

The nucleic acid component of a transfection complex of the invention may, for example, be obtained from natural sources, or may be produced recombinantly or by chemical synthesis.

The nucleic acid component may, for example, consist of or comprise a molecule having a specific function, for example, a nuclear targeting molecule. The nucleic acid may be DNA or RNA. DNA may be single stranded or double stranded. The nucleic acid may be suitable for use in gene therapy, in gene vaccination or in anti-sense therapy. The nucleic acid may be or may relate to a gene that is the target for particular gene therapy or may be a molecule that can function as a gene

vaccine or as an anti-sense therapeutic agent. The nucleic acid may be or correspond to a complete coding sequence or may be part of a coding sequence.

Alternatively, the nucleic acid may encode a protein that is commercially useful, for example industrially or scientifically useful, for example an enzyme; that is pharmaceutically useful, for example, a protein that can be used therapeutically or prophylactically as a medicament or vaccine; or that is diagnostically useful, for example, an antigen for use in an ELISA. Host cells capable of producing commercially useful proteins are sometimes called "cell factories".

In the case of a nucleic acid sequence to be expressed, appropriate transcriptional and translational control elements are generally provided.

The nucleic acid is generally DNA but RNA may be used in some cases, for example, in cancer vaccination. The nucleic acid component may be referred to below as the "plasmid component" or component "D".

Examples of nucleic acids that can be used in gene therapy and/or in gene vaccination include the coding sequence of a protein and the cDNA copy and genomic version thereof, the latter including introns as well as exons, and also the regulatory upstream and downstream sequences. Other useful nucleic acids include sequences involved in repairing genes and in homologous recombination. These can be molecules such as RNA/DNA chimeras (Bandyopadhyay et al., 1999; Cole-Strauss et al., 1996; Kren et al., 1998; Yoon et al., 1996) or DNA oligonucleotides (Goncz et al., 1998). A useful nucleic acid can be a short sequence contained in a plasmid, or another large nucleic acid that mediates integration of plasmids or

nucleic acids, for example, phage integrase (Groth et al., 2000; Olivares et al., 2001; Stoll et al., 2002; Thyagarajan et al., 2000; Thyagarajan et al., 2001) and "Sleeping Beauty" transposons (Yant et al., 2000).

DNA oligonucleotides can be delivered for purposes of antisense regulation (Bachmann et al., 1998; Knudsen and Nielsen, 1997; Mannion et al., 1998; Woolf et al., 1995) or as transcription factor decoys (Ehsan et al., 2001; Ehsan et al., 2002; Mann et al., 1999; Morishita et al., 1995). CpG-rich oligonucleotide sequences may be useful as adjuvants to boost vaccine responses (Krieg et al., 1995).

Another important new class of molecules that can be used in gene therapy includes small interfering RNA. As explained above, RNA interference in mammalian cells has emerged in the last two or three years as an important new approach to the regulation of gene expression, with a high degree of specificity (reviewed Shi 2003). Double-stranded RNA molecules of 20-30 nt in length, known as small interfering RNA (siRNA) molecules, target homologous regions of mRNA. They then activate a conserved pathway that leads to degradation of the mRNA target. The precise mechanism of action of siRNA is under intense investigation but it is clear that the application of siRNA to mammalian cells has the potential to revolutionize the field of functional genomics. The ability to simply, effectively, and specifically down-regulate the expression of genes in mammalian cells holds enormous scientific, commercial, and therapeutic potential.

Currently there is no way to predict an effective siRNA target so screening of numerous sequences is performed and numerous potential molecules may have to be screened. Such screening is most conveniently performed with chemically

synthesised siRNA molecules delivered by non-viral vectors. The use of the transfection complexes of the invention for transfecting siRNA molecules for subsequent screening thus provides benefits of cost-effectiveness as well as greater functionality. siRNA molecules may also be used therapeutically. Such transfection and subsequent screening of SiRNAs is part of the present invention.

The efficiency of transfection of a peptide of the invention, a polycationic nucleic acid-binding component, a peptide derivative of the invention, a lipid component or any combination thereof may be determined readily using the methods described herein.

The efficiency of transfection using a transfection complex as described above as transfection vector is influenced by the ratio lipid component:peptide/polycationic nucleic acid-binding component i.e. peptide derivative of the invention:DNA or RNA. For any chosen combination of components for any particular type of cell to be transfected, the optimal ratios can be determined simply by admixing the components in different ratios and measuring the transfection rate for that cell type, for example, as described herein.

Lipofectin and lipofectamine appear to be particularly effective in enhancing transfection in the system described above. Lipofectin has the advantage that only very small amounts are required. Any side effects that may occur are therefore minimised.

A suitable weight ratio between the lipid and the DNA components has been found to be 0.75:1. For any given transfection experiment, this ratio may be optimised using methods known in the art.

A transfection mixture of the invention may be produced by admixing component (i), the lipid component, component (ii), the polycationic nucleic acid binding component, and component (iii), the peptide of the invention. A transfection complex as described above may be produced by admixing components (i), (ii), (iii) and (iv), the nucleic acid component. Components (ii) and (iii) are preferably in the form of a peptide derivative of the invention.

Although the components may be admixed in any order, it is generally preferable that the lipid component is not added last. In the case where a peptide derivative is used, it is generally preferable to combine the components in the following order: lipid component; peptide derivative; DNA or RNA component.

A transfection mixture comprising a peptide derivative and a lipid component may be used to produce a nucleic acid-containing transfection complex by the incorporation of the nucleic acid with the transfection mixture, for example, by admixture. Alternatively, the transfection mixture may be used for the production of a vector complex which comprises, instead of the nucleic acid component, any other component that is capable of binding to the polycationic nucleic-acid binding component, for example, a protein.

Transfection mixtures of the invention are stable on storage at 4°C. It may therefore be convenient to prepare the transfection mixture in bulk and to use portions of the transfection mixture as and when required to prepare a transfection complex incorporating a nucleic acid of choice.

A transfection mixture of the invention preferably comprises an equimolar mixture of DOPE and DOTMA (lipofectin) as the lipid component, and a peptide of the invention, especially

peptide derivative of the invention, for example, a $[K]_{16}$-peptide. A spacer as described above may be present in the peptide derivative. The preferred molar ratio lipofectine:peptide derivative is 0.75:4.

The individual components of a transfection mixture of the invention are each described herein. The preferred components, preferred combinations of components, preferred ratios of components and preferred order of mixing, both with regard to the transfection mixture and to the transfection complex and its production are described herein.

The invention further provides a non-viral transfection complex comprising:

(i) a nucleic acid,

(iii) a polycationic nucleic acid-binding component, and

(iv) a peptide of the invention.

Cells that may be transfected by a transfection complex comprising a peptide of the invention include, for example, endothelial and epithelial cells, for example, cells of any part of the airway epithelium, including bronchial and lung epithelium, and the corneal endothelium. The airway epithelium is an important target for gene therapy for cystic fibrosis and asthma.

The invention also provides a viral vector, which vector comprises a peptide of the invention.

A viral vector is targeted to the receptor to which the virus binds to gain entry into its host cell. Such a vector can be retargeted by combining a peptide of the invention with the vector. The peptide should be combined in such a manner and at such a site that the vector can bind to host cells and that can still function as a vector.

The viral vector also comprises a nucleic acid of interest. Such a nucleic acid may be as described above in relation to a transfection complex of the invention. The incorporation of a peptide of the invention in a viral vector should not interfere with the nucleic acid. The peptide of the invention may be incorporated with the viral vector before or after the nucleic acid is inserted into the vector.

The viral vector is, for example, an adenovirus, the native receptor for which is the Coxsackie-Adenovirus Receptor (CAR) protein. This is the primary receptor for adenovirus. Secondary receptors include integrins and proteoglycans. CAR is found on many cell types but not on dendritic cells and the apical surface of airway epithelial cells, which restricts the efficiency of adenoviral vectors in the cells and tissues. Adenoviral vectors can be retargeted, for example, by incorporation of a peptide of the invention, for example, in the HI region of the fibre protein in the capsid (Nicklin et al., 2001).

Adenovirus type 5, for example, is a good vector in principle as it transduces a wide range of cell types with high efficiency but is a poor vector for dendritic cells as such cells lack both the CAR and integrin receptors. When a peptide of the present invention is inserted into the HI region of the fibre protein of the capsid, transfection efficiency for dendritic cells increased from 20 to 45% as observed using an adenoviral type 5 vector with a wild type fibre protein in the capsid to between 64 and 79%. However, the increase in transfection efficiency is also observed in other cells, not only dendritic cells. For example, transfection of human primary macrophages using a retargeted adenoviral vector incorporating a peptide of the invention increased from about 13% as observed with wild-type virus to

up to about 67%; with N2a cells the transefection efficiency was about 63% for retargeted vector compared with about 24% for wild type vector; with HAEo cells the values were about 82% compared with 45%; with HMEC cells about 95% compared with 73% and with HepG2 cells about 88% compared with 63%, see Table 5 above.

Alternatively, a viral vector may be retargeted by formation of a complex with a peptide of the invention that comprises a cationic domain that is capable of binding electrostatically to the viral capsid or coat. An example of such a peptide is a peptide derivative of the invention, which comprises a polycationic nucleic acid binding component, for example, a polycationic oligolysine, for example, having from 3 to 32 lysine residue, for example, as described above, for example $[K]_{16}$. An electro-static complex between the virus and the modified peptide may be produced by mixing solutions of the virus and the peptide. Such a complex is part of the present invention.

In a further alternative, a peptide of the invention is incorporated with the viral vector by means of an antibody that is capable of binding to the virus. The antibody may be a bispecific antibody capable of binding to the peptide and to the virus, or the peptide and the antibody may be in the form of a fusion protein. In either case the peptide may be used to mediate adenoviral binding and display on the virus, allowing retargeted transduction (Watkins et al., 1997). The antibody component may be of any antibody class, may be an appropriate antigen-binding domain or domains, and may be or be derived from a chimeric or humanised antibody. Such a fusion protein is part of the present invention. A bispecific antibody and the peptide or the peptide-antibody fusion protein may be contacted with a viral vector and allowed to bind. Methods of making and selecting bispecific

antibodies and peptide/antibody fusion proteins are known in the art, see for example, (Nicklin et al., 2001; Pereboev et al., 2002; Tillman et al., 1999; Watkins et al., 1997; Wickham et al., 1997)

A complex, a bispecific antibody and a peptide-antibody fusion protein are all part of the present invention.

The transfection efficiency of a viral vector of the present invention for any particular cell or cell type relative to the efficiency of a wild type vector may be determined readily, for example, as described in Example 3 below. Although described above in relation to adenovirus, a viral vector of the present invention may be a viral vector that can be retargeted using a peptide of the present invention. Examples of such viral vectors include genetically engineered, replication-defective derivatives of retrovirus, lentivirus, adenovirus, adeno-associated virus (AAV), and herpes simplex virus (HSV).

Unless specified otherwise, a viral vector of the present invention may be used analogously to a transfection complex of the invention, for example, for same purposes.

The present invention also provides a process for expressing a nucleic acid in host cells, which comprises contacting the host cells *in vitro* or *in vivo* with a transfection complex or viral vector of the invention comprising the nucleic acid, and culturing the host cells under conditions that enable the cells to express the nucleic acid.

The present invention further provides a process for the production of a protein in host cells, which comprises contacting the host cells *in vitro* or *in vivo* with a transfection complex or viral vector of the invention that

comprises a nucleic acid that encodes the protein, allowing the cells to express the protein, and obtaining the protein. The protein may be obtained either from the host cell or from the culture medium. Suitable host cells are well know. Examples of suitable host cells include Chinese hamster ovary (CHO) cells, (Castilho et al., 2002), BHK cells (Cruz et al., 2002), 293 cells (Durocher et al., 2002) and insect cells such as Sf9 [Wang, 2000 #1964].

The present invention further provides a method of transfecting cells comprising contacting the cells with a transfection complex or viral vector according to the invention. Such transfection may be carried out in vitro or in vivo. Cells transfected in vitro may, if desired, be administered to a human or non-human animal subject for therapeutic purposes, see below.

The invention further provides cells, transfected with a nucleic acid by a method according to the invention, and also the progeny of such cells.

The present invention also provides a pharmaceutical composition which comprises a transfection complex or viral vector of the invention comprising a nucleic acid in admixture or conjunction with a pharmaceutically suitable carrier. The composition may be a vaccine, in which case it may comprise an adjuvant.

The present invention also provides a method for the treatment or prophylaxis of a condition caused in a human or in a non-human animal by a defect and/or a deficiency in a gene, which comprises administering to the human or to the non-human animal a transfection complex or viral vector of the invention comprising a nucleic acid suitable for correcting the defect or deficiency.

The present invention also provides a method for therapeutic or prophylactic immunisation of a human or of a non-human animal, which comprises administering to the human or to the non-human animal a transfection complex or viral vector of the invention comprising an appropriate nucleic acid.

The present invention also provides a method of anti-sense therapy of a human or of a non-human animal, comprising anti-sense DNA administering to the human or to the non-human animal a transfection complex or viral vector of the invention comprising the anti-sense nucleic acid.

The present invention also provides the use of a transfection complex or viral vector of the invention comprising a nucleic acid for the manufacture of a medicament for the prophylaxis of a condition caused in a human or in a non-human animal by a defect and/or a deficiency in a gene, for therapeutic or prophylactic immunisation of a human or of a non-human animal, or for anti-sense therapy of a human or of a non-human animal.

An alternative to administering a complex or vector of the invention is to administer cells that have been transfected in vitro.

A non-human animal is, for example, a mammal, bird or fish, and is particularly a commercially reared animal.

The nucleic acid, either DNA or RNA, in the transfection complex or viral vector is appropriate for the intended use, for example, for gene therapy, gene vaccination, anti-sense therapy or protein production, see above. The DNA or RNA and hence the transfection complex or viral vector is administered in an amount effective for the intended purpose.

The treatments and uses described above may be carried out by administering the respective transfection complex, viral vector, agent or medicament in an appropriate manner, for example, administration may be systemic, local or topical, depending on the site of the target cells and the intended effect. For example, in the case of airway epithelia. delivery is generally local and topical, and may involve nebulisation or bronchoscopy. For treatment of eyes, administration may be intraocular. For other target systemic administration may required, in which case administration may be by injection, for example, intravenous, intramuscular or intraperitoneal injection.

In a further embodiment, the present invention provides a kit comprising a transfection complex or viral vector of the invention comprising a nucleic acid.

The present invention also provides a kit that comprises the following items: (a) a peptide of the invention; (b) a polycationic nucleic acid-binding component; and (c) a lipid component. Such a kit may further comprise (d) a nucleic acid. Components (a) and (b) are preferably in the form of a peptide derivative of the invention. Alternatively, a kit may comprise components (a), (b) and (d).

Such a nucleic acid may be single-stranded or double stranded and may be a plasmid or an artificial chromosome. The nucleic acid component may be provided by a transfection transfection complex suitable for the expression of the nucleic acid, the vector complex being either empty or comprising the nucleic acid.

The components (a) to (d) kit are, for example, as described above in relation to a transfection vector mixture or

transfection complex.

The polycationic nucleic acid binding component is preferably an oligolysine, as described above. The lipid component is preferably capable of forming a cationic liposome, and preferably is or comprises DOPE and/or DOTMA, for example, an equimolar mixture thereof, or is or comprises DOSPA, for example, a mixture of DOPE and DOSPA, for example in the weight ratio DOPE:DOSPA of 1:3. The ratios between the components are preferably as described above, as is the order of mixing of the components.

A kit generally comprises instructions, which preferably indicate the preferred ratios of the components and the preferred order of use or admixing of the components, for example, as described above. A kit may be used for gene therapy, gene vaccination or anti-sense therapy. Alternatively, it may be used for transfecting a host cell with a nucleic acid encoding a commercially useful protein i.e. to produce a so-called "cell factory".

Targets for gene therapy are well known and include monogenic disorders, for example, cystic fibrosis, various cancers, and infections, for example, viral infections, for example, with HIV. For example, transfection with the p53 gene offers great potential for cancer treatment. Targets for gene vaccination are also well known, and include vaccination against pathogens for which vaccines derived from natural sources are too dangerous for human use and recombinant vaccines are not always effective, for example, hepatitis B virus, HIV, HCV and herpes simplex virus. Targets for anti-sense therapy are also known. Further targets for gene therapy and anti-sense therapy are being proposed as knowledge of the genetic basis of disease increases, as are further targets for gene vaccination. The present invention

enhances the transfection efficiency and hence the effectiveness of the treatment.

Transfection of cells with pro-inflammatory cytokines may be used in cancer immunotherapy, with anti-inflammatory cytokines in the treatment of auto-immune diseases , for example, rheumatoid arthritis and multiple sclerosis.

Cells may be transfected with an anti-angiogenic gene, for example for soluble VEGF-R for treatment of cancer, or with an angiogenic gene, for example, VEGF, for myocardial diseases, or peripheral vascular disease.

Non-viral transfection complexes of the invention may be effective for intracellular transport of very large DNA molecules, for example, DNA larger than 125kb, which is particularly difficult using conventional vectors. This enables the introduction of artificial chromosomes into cells.

Transfection of the airways, for example, the bronchial epithelium demonstrates utility for gene therapy of, for example, respiratory diseases, such as cystic fibrosis, emphysema, asthma, pulmonory fibrosis, pulmonary hypertension and lung cancer.

Cystic fibrosis (CF) is the most common monogenic disorder in the Caucasian population. Morbidity is mainly associated with lung disease. CF is caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator protein (CFTR), a cell membrane channel that mediates secretion of chloride ions. Correction of this defect in the bronchial cells by CFTR gene transfer will correct the biochemical transport defect and, hence, the lung disease. Clinical trials so far have generated encouraging data but highlighted the need for more efficient, non-toxic

vectors.

The enhanced levels of transfection make the method of the invention particularly suitable for the production of host cells capable of producing a desired protein, so-called "cell factories". For long-term production, it is desirable that the introduced nucleic acid is incorporated in the genome of the host cell, or otherwise stably maintained. That can be readily ascertained. As indicated above, the range of proteins produced in this way is large, including enzymes for scientific and industrial use, proteins for use in therapy and prophylaxis, immunogens for use in vaccines and antigens for use in diagnosis.

The present invention is especially useful with a receptor targeted vector complex that is capable of high efficiency transfection. In a preferred embodiment, the vector complex comprises four modular elements; an oligolysine, especially $[K]_{16}$, DNA-binding or RNA-binding element; a peptide of the invention, for example, a peptide described herein; a DNA or RNA sequence, optionally in a plasmid, and optionally regulated by a viral promoter and an enhancing element; the cationic liposome DOTMA/DOPE (lipofectin). The combination of oligolysine-peptide/DNA or RNA complex with the cationic liposome formulation DOTMA/DOPE is a potent combination. Alternatively a DOPE/DOSPA formulation may be used instead of or in addition to a DOTMA/DOPE formulation. The optimisation of variables associated with complex formation and the mode of transfection by LID vector complexes has been demonstrated.

The most important variables in the formation of optimal transfection complexes appear to be the ratio of the three components and their order of mixing.

The invention further provides a method for identifying an siRNA. A panel of siRNA molecules of 20-30 nucleotides length is designed for homology to regions scattered throughout the target gene sequence. SiRNA molecules can be synthesised by commercial sources, e.g. Qiagen, Promega. A cell that expresses the target gene is transfected with the individual siRNA and expression levels quantified by standard, relevant protein assays or mRNA assays.

The invention provides the use of a peptide of the invention to target an entity to a cell or cell type to which the peptide binds. The entity may be a nucleic acid or another molecule, for example, a therapeutically or pharmaceutically active molecule, or a molecule comprising a detectable label.

The following non-limiting Examples illustrate the present invention.

EXAMPLES

EXAMPLE 1: IDENTIFICATION OF PEPTIDE MOTIFS

MATERIALS AND METHODS

(i) The Peptide Library

The library used, C7C, was obtained commercially from New England Biolabs Inc. Phage growth, titration and amplification procedures were performed as described in the manufacturer's handbook. The library consists of random peptide sequences seven residues in length and flanked by cystine residues to allow cyclisation by oxidation in air. Constrained cyclic peptides often display higher binding affinities than their linear counterparts.

(ii) Generation of immature human dendritic cells (DCs)

Immature dendritic cells were generated from peripheral blood monocytes as described below

10 mls of peripheral blood were diluted 1:1 with Hanks Buffered salt solution (HBSS) then layered on Lymphoprep (Nycomed (UK) Ltd, Sheldon , Birmingham), centrifuged at 750g for 30 min and lymphocytes were isolated from the interface. Excess Lymphoprep was removed by spinning the cells down at 250g for 10 min, and any platelets present were removed by washing in HBSS twice, centrifuging the cells at 1200 rpm for 10 min each time. Monocytes were then isolated using MACs CD14 magnetic microbeads (Miltenyi Biotec Ltd., Bisley, Surrey) as described in the manufacturer's protocol. Monocytes were seeded at 1 x 10^6 cells per well in a 6 well plate, in 3 ml per well complete medium (HEPES buffered RPMI + 10 % fetal calf serum (FCS), 100 U/ml penicillin and 100 µg/ml streptomycin) supplemented with recombinant human GM-CSF (Granulocyte-macrophage colony-stimulating factor) (Leucomax)(purchased from Schering Plough/ Sandoz, Innishannon, Ireland) and IL-4 (obtained from PreproTech EC Ltd, London,UK) cytokines (100 ng/ml and 25 ng/ml final concentrations, respectively) and incubated at 37°C, 5 % CO_2. Fresh cytokines were added on day 3 of incubation and the cells were used on day 6.

(iii) Dendritic cell "panning" in solution

Selection of dendritic cell binding phage from the peptide library, was carried out as below using approximately 1 x 10^6 viable dendritic cells obtained by the method described in Example 1(ii) above per round in solution, and using 2 x 10^{11} phage from the C7C library, see Example 1(i) above.

Before addition of the phage library to the cells, both were blocked for 30 min, each in 1ml 2% Marvel (dried milk) 5% BSA-PBS at 4°C in an up-and-over turntable. The phage were

allowed to bind to the cells for 2 hours with mixing, before centrifuging the cells at 2000 rpm for 3 min to remove the supernatant, which contains non-binding phage. The cells were then washed five times in 2% BSA-PBS, for five minutes each, transferring the cells to clean tubes each time to remove non-specific or weakly binding phage. Two washes in PBS only were then carried out, followed by elution using 76mM citrate buffer pH 2.5 for 10 min with mixing in an up-and-over turntable. The cells were spun down, and the eluate removed, neutralised by the addition of 600µl 1M Tris pH 7.5, and stored at 4°C (the 'eluted fraction'). The remaining cells were lysed with 1ml 30mM Tris pH 8.0, 1mM EDTA for 1 hour on ice, before vortexing briefly & storing at 4°C for a phage titration later, see Example 1(iv)(b) below.

Harvested phage from each cell lysis fraction were amplified in *E.coli ER2738* (New England Biolabs (UK) Ltd, Hitchin, Herts) as described in the manufacturer's instructions, titred and used as the input phage for the next round of panning. Three rounds of panning were carried out using dendritic cells from two different donors for each round to avoid isolating HLA-specific binding peptides. All panning was carried out at 4°C or below to prevent alterations in the dendritic cell phenotype. For the second and third rounds of panning, the wash stringency was reduced to three times with 2% BSA-PBS and twice with PBS only because of the low numbers of phage harvested.

(iv) Characterisation of cell binding of selected phage

Binding of phage to human monocyte-derived immature dendritic cells (DCs) was investigated by whole cell flow cytometry and phage titration assays.

(a) Whole cell flow cytometry

On days, non-adherent immature human DCs produced as described in Example 1(ii) above were harvested. 5×10^4

Dendritic cells and 2 x 10^{11} of an individual purified phage clone were each blocked at 4°C for 30 mins in 1ml MBSA (2% Marvel, 5% BSA in PBS), with mixing on a turntable. The dendritic cells were spun down at 2000 rpm, a gentle centrifugation to pellet cells without lysing them, for 5 min at 4°C, and the pellet resuspended in the blocked phage solution. The mixture was held on ice for 30 min to allow the phage to bind, before spinning down the cells, removing the supernatant and washing the cells with 1ml 1% BSA, 0.05% Tween20-PBS. The cells were spun again, the supernatant removed, and the cells fixed in 1ml 1% paraformaldehyde for 30 min on ice. The cells were washed twice in PBS, resuspended in 1ml 1% Marvel, 2.5% BSA in PBS and 2µl of FITC(fluorescein-isothiocyanate)-labelled anti-Fd antibody (Sigma, Poole, Dorset)(3mg/ml) added. The cells were incubated with the antibody on ice for 30 min before spinning down, washing twice in 0.05% Tween20, 1% BSA PBS, resuspending in 200µl PBS and measuring the percentage cells positive for FITC by FACs analysis.

(b) Titration of phage clone binding to cells

For each titration 5 x 10^4 monocyte derived-dendritic cells (day 5) were initially blocked by incubating in 200µl blocking buffer (DMEM/2% Marvel/1% BSA) for 30 min at 4°C, mixing constantly. 2x 10^{11} Phage were also blocked in 200µl blocking buffer for 30 min at 4°C. The dendritic cells were spun down at 2000 rpm, for 5 min at 4°C, and resuspended in the phage solution, allowing the phage to bind for 1 hour on ice with occasional mixing. The cells were then washed in PBS-Tween 20 (0.05%) three times before eluting phage with 166µl TBS pH5.5. for 10 min on ice. The eluate was neutralised with 34µl 1M Tris-HCl pH 8, the cells were spun down, and the supernatant removed to be stored as eluate. The cell pellet was resuspended in 200µl of cell lysis buffer

shaking. The cell debris was removed by spinning at 5000 rpm for 5 min and the supernatant recovered. The phage in the eluate and the cell lysate were titred by measuring plaque forming units (pfu) in *E.coli* ER2738 as described in the NEB C7C technical bulletin.
The titration of phage clone binding was carried out using human and mouse dendritic cells, with binding to plastic as a control. The human cells were obtained from donors JD, S and LA.

(c) Sequencing phage peptide inserts
81 Individual phage clones isolated from the cell lysis fraction following the third round of dendritic cells selection were purified from small scale polyethylene glycol (PEG) preparations (see NEB C7C technical bulletin, and single stranded phage DNA was prepared for sequencing [Kay, 1996 #50].

Briefly, the protein coat was removed from the sample by phenol chloroform extraction, and the DNA pelleted by ethanol precipitation. Trace salt was washed from the pellet with ice cold 70% ethanol before resuspending the DNA in Tri-EDTA (TE). From 50 to 100 ng purified DNA was used in a Big Dye (Applied Biosystems, Foster City, California, USA) terminator cycle sequencing reaction (following the manufacturers instructions) using the -96 primer (5'-CCCTCATTAGCGTAACG-3') (supplied with the C7C library) and purified for loading by ethanol precipitation as described in the kit instructions. The samples were run on an ABI 377 sequencer (Applied Biosystems, Foster City, California, USA) and the results were analysed using the Vector NTI Suite of programs (Informax Inc, Oxford UK).

RESULTS

Peptide Identification

Phage were recovered and titred from each round of selection as follows. $2x10^{11}$ Blocked phage were added to 5×10^4 blocked monocyte-derived immature dendritic cells for 1 hour on ice before washing cells three times with 0.05% Tween20-PBS, eluting phage with TBS pH5.5, and lysing cells to harvest the phage remaining bound. The numbers of phage harvested by cell lysis were calculated as plaque forming units (pfu). Figure 1 shows results of some of the titrations. Sequencing of the bound phage shown in Figure 1 identified the peptides as APSNSTA, QLLTGAS, TARDYRL, FQSQYQK, PLMPSLS, FPRAPHH, MASISMK, DWWHTSA, SHVKLNS, and SPALKTV.

Sequencing of 81 phage clones from the cell-associated fraction from the third round of titration of phages clone binding to immature dendritic cells identified 16 different sequences, see Table 2.

TABLE 2 Phage sequences from third round of tittration of phage clone binding to immature dendritic cells.

Sequence	Number of clones	Percentage of clones
APSNSTA	17	21
DWWHTSA	16	20
SHVKLNS	10	12
SQKNPQM	6	7
QLLTGAS	5	6
SPALKTV	5	6
FQSQYQK	5	6
TARDYRL	4	5

FPRAPHH	4	5
STPPNTT	3	4
PMLPSLS	1	1
SEWLSAL	1	1
IGGIRRH	1	1
YTMEFNR	1	1
MASISMK	1	1
PAAYKAH	1	1

The three most frequent phage clones are present at 21% (APSNSTA), 20% (DWWHTSA) and 12% (SHVKLNS) with the remainder present at 7% and below. Analysis of the 16 binding sequences from the phage clones identified five minimal motifs, namely, $PXN^{T}/_{S}T$, $PXXXT^{A}/_{V}$, $^{A}/_{L}PSXS$, $S^{L}/_{I}S$, and $QX^{N}/_{Q}XQ$, see Table 2B, which motifs may play an important role in binding to receptors on dendritic cells. Of all the clones sequenced, 46% contained one or more motifs, with the most frequent clone, APSNSTA, showing a degree of homology to three other peptide sequences, see Table 3.

TABLE 3 Conserved amino acid motifs in peptide sequences

Peptide Homology	Motif	% clones containing motif
A*P*SNS*TA* S*P*ALK*TV*	$PXXXT^{A}/_{V}$	27
ST*P*P*N*T*T* A*P*S*N*S*T*A	PXNXT	25
*APS*N*S*TA PM*LPS*L*S*	$^{A}/_{L}PSXS$	22
S*Q*K*N*P*Q*M F*Q*S*Q*Y*Q*K	$QX^{N}/_{Q}XQ$	13
PMLP*S L S* MA*S IS*MK	$S^{L}/_{I}S$	2

Identical amino acids are shown in bold and italic
Similar amino acids are shown in italic only

Titrations of phage clone binding to dendritic cells in most cases showed that the clones bind to a greater extent to the cells than do phage that have no insert in the cells. Two clones, FPRAPHH and MASISMK bound in highest numbers in all titrations, including the titration of phage binding to mouse dendritic cells. The numbers of phage binding to plastic was low for all clones tested, suggesting that phage binding demonstrated by high titres in these experiments is due to binding to cells and not background non-specific binding to the wells or blocking molecules, see Figure 1 and Table 6. .

TABLE 6. Titration of phage binding to human dendritic cells, mouse dendritic cells and blocked plastic

Peptide displayed on phage	human donor JD	Human donor S	Human donor LA	Mouse DCs	Plastic
APSNSTA	460	6480	1920	480	480
QLLTGAS	960	220	380	20	220
TARDYRL	180	940	N/D	N/D	N/D
FQSQYQK	360	3060	1000	3260	1680
PMLPSLS	40	560	40	1520	80
FPRAPHH	3200	19400	15920	13980	1860
MASISMK	7780	9440	15220	6380	2380
DWWHTSA	1380	8020	2980	3240	1180
SHVKLNS	1160	7280	2620	2120	720
SPALKTV		6960	120	600	220
None	1400	240	80	100	5.6

Figures shown x 10^3

FACs analysis of phage binding to dendritic cells from five different dendritic cell donors (SB, U, SH, KG and AM) with six of the most frequent clones namely APSNSTA, FQSQYQK,

DWWHTSA, SHVKLNS, SPALKTV, and SQKNPQM showed that all clones except for one, SPALKTV, were detected binding to a higher percentage of cells than a phage clone bearing no insert, see Figure 2.

The pattern of binding identified the three clones binding to dendritic cells in highest amounts as those containing the peptides APSNSTA,DWWHTSA and SHVKLNS, which clones were also the three most frequently isolated from the selection. For the second set of six clones tested, namely those containing the peptides QLLTGAS, TARDYRL, PMLPSLS, FPRAPHH, MASISMK, and STPPNTT, all clones showed a higher percentage of cells positive for bound phage than the controls with no insert. QLLTGAS binds to marginally more cells than the others, see Figure 3, in which the dendritic cell donors are KG and SB.

Of the 16 phage sequences two, namely APSNSTA and SHVKLNS were chosen for synthesis on the basis that they were amongst the most frequent clones and also were two of the top binders in the FACs assay of phage clone binding. SHVKLNS is called "peptide A" and APSNSTA is called "peptide B".

EXAMPLE 2: NON-VIRAL TRANSFECTION

MATERIALS AND METHODS

(i) Peptide synthesis

Peptides A and B, identified from phage that display desirable cell binding and entry characteristics, see Example 1, were synthesised using standard synthetic chemistry with a sixteen-lysine tail, a GAC linker, and a C-terminal CG group. Peptide 6, RRETEWA, is an integrin-binding peptide. Peptide 6J, ATRWARE, is a scrambled version of peptide 6, and serves as a control peptide. The term "peptide derivative" is used

to denote the synthesized peptide sequence shown below i.e. "peptide A" denotes SHVKLNS and "peptide A derivative" denotes $[K]_{16}$-GACSHVKLNSCG. Details of the peptides and peptide derivatives are given in Table 7 below.

TABLE 7

Peptide name	Peptide sequence on phage	Peptide derivative sequence
A	CSHVKLNSC	$[K]_{16}$-GACSHVKLNSCG
B	CAPSNSTAC	$[K]_{16}$-GACAPSNSTACG
6	CRRETEWAC	$[K]_{16}$-GACRRETEWACG
6J		$[K]_{16}$-GACATRWARECG

(ii) Formation of lipopolyplex transfection complexes and transfection of immature DCs

(a) Transfection complexes for immature DCs

Transfection complexes comprising lipid, peptide and DNA were prepared. The lipid component was lipofectin (Invitrogen Ltd, Paisley, UK), the peptide component was a synthesized peptide A, B, 6 or 6J derivative, as described in the Table in Example 2(i) above, and the DNA was the plasmid pEGFP-N1 (Clontech, BD Biosciences, Palo Alto, CA)

In the transfection complex, peptide component to DNA charge ratios (+/-) were used at 1.5:1, 3:1 and 7:1. The lipid component was maintained at a constant proportion, by weight, relative to DNA of 0.75:1. Prior to making transfection complexes the lipid component was diluted to a concentration of 15 µg per ml, the peptide was prepared at 0.1 mg/ml and the DNA was at 10 µg per ml. All dilutions were performed with OptiMEM reduced serum tissue culture medium (Life

Technologies Ltd, Paisley, UK). Transfection complexes were made by mixing the components in the order 1) lipid then 2) peptide and finally 3) DNA, then diluted with OptiMEM to a concentration relative to the DNA component of 2μg DNA per 300μl.

(b) Transfection of immature DCs

Immature day 6 dendritic cells, obtained as described in Example 1(ii) above, were plated into a 48 well plate at 5 x 10^4 cells per well in complete media and allowed to settle for 3 hours at 37°C. 300μl of the transfection complex obtained as described above was added to each well. The transfection complex was applied to cells within 5 minutes of preparation. Transfection incubations were performed at 37°C for 4 hours, after which the medium was replaced with complete medium with cytokines for 24 hours. Cells were harvested by scraping, spun down and resuspended in 300μl PBS for FACs analysis to determine the percentage of cells positive for the reporter gene EGFP (Enhanced Green Fluorescent Protein). Each transfection was performed in triplicate wells.

(iii) Transfection of other cell types

(a) Other cell types

The other cells types used were HMEC-1 cells (CDC, Atlanta, GA 30333, U.S.A), HAEo- cells (courtesy DC Gruenert, Human Molecular Genetics Unit, Department of Medicine, University of Vermont, Burlington, VT 05405, USA) and N2a cells (LGC Promochem, Teddington, Middlesex, UK).

(b) Transfection complexes for other cell types

Transfection complexes comprising lipid, peptide and DNA were prepared. The lipid component was lipofectin (Invitrogen Ltd, Paisley, UK), the peptide component was a peptide A, B,

6 or 6J derivative, as described above, and the DNA was the plasmid pCILuc (Promega UK Ltd, Southampton). In the transfection complex, peptide to DNA charge ratios (+/-) were used at 3:1, 5:1 and 7:1. The lipid component was maintained at a constant proportion, by weight, relative to DNA of 0.75:1. Prior to making transfection complexes the lipid component was diluted to a concentration of 15 µg per ml, the peptide was prepared at 0.1 mg/ml and the DNA was at 40 µg per ml. All dilutions were performed with OptiMEM reduced serum tissue culture medium (Life Technologies). Transfection complexes were made by mixing of components in the order 1) lipid then 2) peptide and finally 3) DNA, then diluted with OptiMEM to a concentration relative to the DNA component of 0.25µg DNA per 200µl.

(c) Transfection of other cell types

For transfection of these types of cell i.e. HMEC, HAEo- and N2a, 1 x 10^4 cells were seeded overnight in 96 well plate. 200□l of the suspension of the transfection complex was added to each well. The suspension was applied to cells within 5 minutes of preparation. Transfection incubations were performed at 37°C for 4 hours, after which the medium was replaced with the appropriate complete medium for 24 hours. Each transfection was performed in replicates of six.

Luciferase reporter gene assays in cell free extracts were performed after incubation for 24 hours using the luciferase assay from Promega UK Ltd using the manufacturer's protocol. Light units were standardised to the protein concentration within each extract.

RESULTS

The two constrained peptides synthesised with a DNA-binding $[K]_{16}$ domain, i.e. the peptide A derivatives $[K]_{16}$-

GACSHVKLNSCG and the peptide B derivative $[K]_{16}$-GACAPSNSTACG, were tested for their ability to transfect immature dendritic cells in a lipopolyplex with DNA and lipid. The results are shown in Figure 4.

Transfection efficiency, as measured by the percentage cells positive for the reporter gene EGFP determined by FACs, was increased by the use of the phage derived peptide A (SHVKLNS) to a level approximately one and a half times that of the positive control, peptide 6, the integrin binding peptide RRETAWA and the negative control, peptide 6J, (the scrambled version of peptide 6). Peptide B (APSNSTA) produced transfection levels equal to those of peptide 6. The percentage of cells transfected did not reach 5%, possibly due to the toxic effect of the transfection procedure on the dendritic cells.

For the other cell types, the transfection efficiency was measured by luciferase activity per mg of protein present. In all cell lines tested, namely HMEC-1, HAEo- and N2a cells, at least one of the peptides A and B produced transfection efficiencies equal to or above those seen with peptide 6.

In HMEC-1 cells, whilst peptide A produces a transfection efficiency approximately equal to that seen with peptide 6, peptide B can increase efficiency by one and a half times that of peptide 6, see Figure 5.

In HAEo- cells, peptide A produced the highest efficiency of transfection, approximately double that seen with peptide 6, whilst efficiency using peptide B was one and a half times that using peptide 6, see Figure 6.

In N2a cells, only peptide A produced transfection efficiencies equal to those seen with peptide 6, with peptide

B producing efficiencies of less than half that value, see Figure 7.

EXAMPLE 3: RETARGETING ADENOVIRUS

MATERIALS AND METHODS

(i) Retargeted adenovirus construction and production

Retargeted adenovirus type 5 having peptide A (SHVKLNS) or peptide B (APSNSTA) inserted into the HI region of the fibre protein of the capsid were constructed and produced in the laboratory of Dr Dan von Seggern, Scripps Research Institute, California, USA using the method described in Nicklin 2001 Mol Ther 2001 Dec;4(6):534-42.

(ii) Transduction using adenovirus

Retargeted adenoviruses carrying peptide A or peptide B were used for the transduction experiments described below. Wild type adenovirus type 5 and type 5 adenovirus having a KO1 fibre, all supplied by Dr Dan von Seggern, and as described in Nicklin 2001 Mol Ther 2001 Dec;4(6):534-42), were used as controls.

(a) Transduction of dendritic cells

Day 5 dendritic cells (see Example 1(ii) above) were plated at 5 x 10^4 cells per well in a 48 well plate in complete medium and allowed to settle for 3 hours at 37°C. Transductions were carried out in complete media, with virus being added at 100,000 viral particles/cell for 24 hours. Cells were harvested by scraping, spun down at 2000 rpm for 5 min, resuspended in 300μl PBS, and kept on ice before analysis. Viral transduction as determined by percentage of GFP positive cells was measured by FACs analysis. To measure percentage viability, 10μl of 7-amino-actinomycin D (7AAD; Sigma, Poole, Dorset) was added immediately prior to analysis. Cells were pooled from three separate transduction experiments for the calculation of results.

(b) Transduction of human primary macrophages

Primary macrophages were generated from peripheral blood monocytes as described below.

10 mls of peripheral blood were diluted 1:1 with HBSS then layered on Lymphoprep (Nycomed), spun at 750g for 30 min and purified lymphocytes were isolated from the interface. Excess Lymphoprep was removed by spinning cells down at 250g for 10 min and any platelets present were removed by washing in HBSS twice, spinning the cells at 1200 rpm for 10 min each time. Monocytes were then isolated using MACs CD14 microbeads as described in the manufacturer's protocol. The mononuclear cells were plated into a $25cm^3$ flask in 10% FCS, RPMI for 1-2hours (1 flask per 10mls blood) before removing the supernatant and adding fresh 10% FCS, RPMI + 10ng/ml MCSF (macrophage colony stimulating factor). Half the medium was replaced with fresh 10% FCS, RPMI + MCSF (10ng/ml) after 48 hours and the cells were used at day 6, harvesting by scraping the cells from the well.

Viral transduction was carried out at 10,000 particles/cell in medium containing 2.5% FCS. Viral transduction as determined by percentage of GFP positive cells was measured by FACs analysis. Cells were pooled from three separate transduction experiments for the calculation of results.

(c) Transduction of other cell types

N2a cells, HAEo- cells, HMEC-1 cells and HepG1 cells were tranduced with the retargeted adenovirus. The N2a, HMEC-1 and HAEo- cells were obtained from the sources described in Example 2(iii)(a) above. The HepG1 cells were obtained from LGC Promochem, Teddington, Middlesex, UK.

For transduction of these other cell types, cells were seeded overnight at 5x 10^4 cells per well in 24 well plates in the appropriate media. Adenovirus was added in OptiMEM at 10,000 viral particles/cell, 1ml per well in triplicate and incubated for 24 hrs at 37°C. Cells were then washed twice in PBS, trypsinised, triplicate wells pooled, spun down at 1200 rpm for 5 min and resuspended in 300µl PBS before analysis by FACs. Where cell viability was to be measured, 10µl 7-amino-actinomycin D (7AAD; Sigma, Poole, Dorset) was added just prior to analysis. Cells were pooled from three separate transduction experiments for the calculation of results.

RESULTS

Gene transfer of retargeted EGFP-reporter gene-bearing adenoviral constructs to immature dendritic cells from two different donors at 100,000 viral particles/cell in complete medium was measured by FACs. In both donors, retargeting the adenovirus with either peptide A or peptide B produced a transduction efficiency of between 64 and 79%, both being of similar efficiencies, and both transducing a significantly higher percentage of cells compared with adenovirus with a wild type fibre protein in the capsid, (between 20 and 46% positive), a KO1 fibre protein (between 0.7 and 1.4 %), and a fibre protein bearing an irrelevant peptide (between 12 and 43 %), see Table 8 and Figure 8. No significant toxicity was seen in any transduction, with cell death being measured at between 5 and 15%.

TABLE 8 Transduction of human monocyte-derived immature dendritic cells

Percentage cell death shown in brackets

Donor	Wild type	KO1	Irrelevant	A	B
A	46.0 (5.7)	1.4 (6.8)	12.2 (9.4)	64.2 (12.0)	65.1 (6.6)
B	20.5 (14.5)	0.7 (13.7)	43.1 (12.1)	78.9 (13.3)	78.9 (11.6)

Transduction of human primary macrophages at 10,000 viral particles/cell in 2.5% serum also demonstrated that incorporating either peptide A (67.6% cells transduced) or peptide B (34.6% of cells transduced) into the viral coat significantly increased the efficiency of transduction above that seen with virus bearing a wild type fibre protein (13.3%) or KO1 fibre protein (9.2% cells transduced), see Table 4.

TABLE 4 Transduction of human monocyte-derived primary macrophages

	Wild type	KO1	Irrelevant	A	B
Primary human macrophages	13.3	9.2	N/D	67.6	34.6

In all other cell types, when virus was added at 10,000 particles per cell in OptiMEM, virus bearing peptide A or peptide B resulted in significantly higher transduction

efficiency than virus bearing wild type fibre protein, KO1 fibre protein or fibre protein bearing an irrelevant peptide, see Table 5

TABLE 5. Transduction of other cell types

Cell type	Virus				
	Wild type	KO1	Irrelevant	A	B
N2a	24.3	1.4	1.3	63.5	53.7
HAEo-	45.8	2.0	3.2	82.9	79.0
CdcHMEC-1	73.3	2.7	25.1	95.7	94.2
HepG2	63.8	1.7	9.7	88.5	79.7

In N2a cells, virus bearing peptide A produced 63.5% tranduced cells, peptide B 53.7%, whilst wild type produced 24.3, KO1 1.4% and irrelevant peptide 1.3%.

In HAEo- cells, peptide A produced 82.9% transduced cells, peptide B 79%, whereas wild type fibre bearing virus transduced 45.8%, KO1 virus 2% and virus bearing an irrelevant peptide 3.2%.

The same patterns of transduction efficiencies were seen with HMEC cells (peptide A 95.7%, peptide B 94.2%, wild type 73.3, KO1 2.7% and irrelevant 25.1%) and HepG2 cells (peptide A 88.5%, peptide B 79.7%, wild type 63.8%, KO1 1.7% and irrelevant 9.7%).

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6.6.03

CLAIMS:

1. A peptide consisting of or comprising an amino acid sequence selected from

a) $PX^1X^2X^3T$ [SEQ.ID.NO.:1];
b) PSX^4S [SEQ.ID.NO.:2];
c) $QX^5X^6X^7Q$ [SEQ.ID.NO.:3];
d) SX^8S [SEQ.ID.NO.:4],

in which X^1, X^2 and X^3, which may be the same or different, each represents an amino acid residue;
X^4 represents an amino acid residue; and
X^5 and X^7, which may be the same or different, each represents an amino acid residue, X^6 represents an amino acid residue having an amide side chain; and
X^8 represent an amino acid having an aliphatic side chain.

2. A peptide as claimed in claim 1 consisting of or comprising an amino acid sequence $PX^1X^2X^3T$ [SEQ.ID.NO.:1] in which X^2 represents N or L.

3. A peptide as claimed in claim 2, wherein X^2 represents L.

4. A peptide as claimed in claim 3, wherein X^1 represent S, A or P.

5. A peptide as claimed in claim 3 or claim 4, wherein X^3 represents S, K or T.

6. A peptide as claimed in any one of claims 3 to 5, wherein X^1 represents A.

7. A peptide as claimed in any one of claims 3 to 6,

wherein X^3 represents T.

8. A peptide as claimed in claim 3, consisting of or comprising the amino acid sequence PALKT.

9. A peptide as claimed in claim 1 or claim 2, wherein X^2 represents N.

10. A peptide as claimed in claim 9, wherein X^1 represent S or P.

11. A peptide as claimed in claim 9 or claim 10, wherein X^3 represents S or T.

12. A peptide as claimed in claim 1, consisting of or comprising the amino acid sequence PALKT [SEQ.ID.NO.:6], PSNST [SEQ.ID.NO.8], or PPNTT [SEQ.ID.NO.:9].

13. A peptide as claimed in any one of claims 1 to 12, having an A or V residue at the C-terminus.

14. A peptide as claimed in any one of claims 1 to 13, having an A, S or T residue at the N-terminus.

15. A peptide as claimed in claim 12 or claim 13, having the sequence APSNST [SEQ.ID.NO.:X], SPALKT [SEQ.ID.NO.:X], TPPNTT [SEQ.ID.NO.:X], STPPNTT [SEQ.ID.NO.:X], and APSNST[SEQ.ID.NO.:X], APSNSTA [SEQ.ID.NO.:X], SPALKTV [SEQ.ID.NO.:X], and APSNSTA [SEQ.ID.NO.:X].

16. A peptide as claimed in claim 1, consisting of or comprising an amino acid sequence PSX^4S [SEQ.ID.NO.:2], in which X^2 represents N or L.

17. A peptide as claimed in claim 16, having an A or L

residue at the N-terminus.

18. A peptide as claimed in claim 17, having the sequence LPSLS [SEQ.ID.NO.:X].

19. A peptide as claimed in any one of claims 16 to 18, having one or more further residues at the N-terminus.

20. A peptide as claimed in claim 19, having the sequence MLPSLS SEQ.ID.NO.:X] or PMLPSLS [SEQ.ID.NO.:X].

21. A peptide as claimed in claim 1 consisting of or comprising an amino acid sequence $QX^5X^6X^7Q$ [SEQ.ID.NO.:3] in which, independently, X^6 represents an N or Q residue.

22. A peptide as claimed in claim 21, wherein independently, X^5 represents K or S and X^7 represents P or Y.

23. A peptide as claimed in claim 21 having the sequence QKNPQ [SEQ.ID.NO.:] OR QSQYQ [SEQ.ID.NO.:].

24. A peptide as claimed in any one of claims 21 to 23 having an S or F residue at the N-terminus and/or an M or K residue at the C-terminus.

25. A peptide as claimed in claim 24 having the sequence SQKNPQM [SEQ.ID.NO.:] or FQSQYSQK [SEQ.ID.NO.:].

26. A peptide as claimed in claim 1 consisting of or comprising an amino acid sequence SX^8S [SEQ.ID.NO.:4] in which X^8 represents L or I.

27. A peptide as claimed in claim 26, having, independently either or both of an A or P residue at the N-terminus and an M residue at the C-terminus.

28. A peptide as claimed in claim 27, having one or more further residues at the N- terminus and/or C- terminus.

29. A peptide as claimed in claim 27 or claim 28, having the sequence PMLPSLS or MASISMK or a variant thereof in which one or more of the terminal residues are omitted.

30. A peptide as claimed in claim 1 and as describe in Table 1 herein.

31. A peptide as claimed in claim 1, having one or more amino acid residues at the N-terminus and/or at the C-terminus.

32. A peptide as claimed in any one of claims 1 to 30 having up to 30 amino acids, for example, having up to 20 amino acids, for example, up to 12 amino acids, for example, having 7 amino acids.

33. A peptide as claimed in any one of claims 1 to 32 comprising a cyclic region of amino acids.

34. A peptide as claimed in claim 33 wherein the peptide comprises two or more cysteine residues capable of forming one or more disulphide bond(s).

35. A peptide as claimed in any one of claims 1 to 34 wherein the peptide is linked to a polycationic nucleic acid-binding component.

36. A peptide as claimed in claim 35 wherein the polycationic nucleic acid-binding component is polyethylenimine or a dendrimer.

37. A peptide as claimed in claim 35, wherein the

polycationic nucleic acid-binding component is an oligopeptide comprising one or more cationic monomers.

38. A peptide as claimed in claim 37, wherein the oligopeptide is an oligolysine, an oligoarginine, an oligohistidine, or a mixed oligomer comprising any combination of histidine, arginine and lysine residues.

39. A peptide as claimed in claim 37 or claim 38, wherein the cationic oligopeptide has from 5 to 25 monomers, preferably from 10 to 20 monomers.

40. A peptide as claimed in claim 39, wherein the oligopeptide is oligolysine.

41. A peptide as claimed in claim 40, wherein the oligolysine has from 14 to 18, for example, 6, monomers.

42. A peptide as claimed in any one of claims 35 to 41, wherein the peptide is linked to the polycationic nucleic acid-binding component via a spacer element.

43. A peptide as claimed in claim 42, wherein the spacer element is GG or GA or is longer and/or more hydrophobic than the dipeptide spacers GG (glycine-glycine) and GA (glycine-alanine).

44. A peptide as claimed in claim 42 or claim 43, wherein the spacer element is GA.

45. A peptide as claimed in claim 42, wherein the spacer element is a chemical bond.

46. A peptide as claimed in claim 36, wherein the peptide is linked to the polyethylenimine via a disulphide bond.

47. A peptide derivative of formula A-B-C wherein
A is a polycationic nucleic acid-binding component,
B is a spacer element, and
C is a peptide as claimed in any one of claims 1 to 34.

48. A peptide derivative as claimed in claim 47, wherein the polycationic nucleic acid-binding component is as defined in any one of claims 336 to 41.

49. A peptide derivative as claimed in claim 47 or claim 48, wherein the spacer element is as defined in any one of claims
42 to 46.

50. A peptide derivative as claimed in claim 47, which derivative is in the form of a peptide as claimed in any one of claims 35 to 46.

51. A non-viral transfection mixture that comprises
(ii) a lipid component,
(iii) a polycationic nucleic acid-binding component, and
(iv) a peptide as claimed in any one of claims 1 to 34.

52. A mixture as claimed in claim 51, wherein the polycationic nucleic acid-binding component is as defined in any one of claims 36 to 41.

53. A mixture as claimed in claim 51, wherein components (iii) and (iv) are in the form of a peptide or peptide derivative as claimed in any one of claims 39 to 50.

54. A mixture as claimed in any one of claims 51 to 53, wherein the lipid component is or comprises one or more lipids selected from cationic lipids and lipids having

membrane destabilising or fusogenic properties.

55. A mixture as claimed in claim 54, wherein the lipid component is or comprises the neutral lipid dioleyl phosphatidyl-ethanolamine (DOPE) or a lipid having similar membrane destabilising or fusogenic properties.

56. A mixture as claimed in claim 54 or claim 55, wherein the lipid component is or comprises the cationic lipid N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA) or a lipid having similar cationic properties.

57. A mixture as claimed in claim 56, wherein the lipid component is or comprises a mixture of DOPE and DOTMA, especially an equimolar mixture thereof.

58. A mixture as claimed in claim 57, which comprises an equimolar mixture of DOPE and DOTMA as the lipid component, a peptide as claimed in any one of claims 1 to 34, and $[K]_{16}$ as the polycationic nucleic acid-binding component.

59. A mixture as claimed in claim 57 or claim 58, wherein the ratio lipid component: peptide/polycationic nucleic acid-binding component is 0.75:4 by weight or 0.5 nmol:1.25 nmol on a molar basis.

60. A mixture as claimed in any one of claims 51 to 59, wherein the lipid component is or comprises 2,3-dioleyloxy-N-[2-(spermidinecarboxamido)ethyl]-N,N-dimethyl-1-propanaminium-trifluoridoacetate (DOSPA) or a lipid having similar properties to those of DOSPA.

61. A mixture as claimed in claim 60, wherein the lipid component is or comprises a mixture of DOPE and DOSPA, especially a mixture of one part by weight DOPE to 3 parts by

weight DOSPA.

62. A mixture as claimed in claim 61, which comprises a mixture of DOPE and DOSPA as the lipid component, a peptide as claimed in any one of claims 1 to 14, and $[K]_{16}$ as the polycationic nucleic acid-binding component.

63. A mixture as claimed in claim 62, wherein the ratio lipid component:peptide/polycationic nucleic acid-binding component is 12:4:1 by weight.

64. A process for the production of a mixture as claimed in any one of claims 51 to 63, which comprises admixing components (ii), (iii) and (iv).

65. A non-viral transfection complex that comprises
(i) a nucleic acid,
(ii) a lipid component,
(iii) a polycationic nucleic acid-binding component, and
(iv) a peptide as claimed in any one of claims 1 to 34.

66. A complex as claimed in claim 65, wherein the polycationic nucleic acid-binding component is as defined in any one of claims 36 to 41.

67. A mixture as claimed in claim 65, wherein components (iii) and (iv) are in the form of a peptide or peptide derivative as claimed in any one of claims 39 to 50.

68. A complex as claimed in any one of claims 65 to 67, wherein the lipid component is as defined in any one of claims 54 to 63.

69. A complex as claimed in any one of claims 65 to 68, wherein the nucleic acid component is or relates to nucleic

acid sequence suitable for gene therapy, gene vaccination or anti-sense therapy, or encodes a desired protein.

70. A complex as claimed in any one of claims 65 to 69, wherein the nucleic acid component is the coding sequence of a protein or the cDNA copy or genomic version thereof, the latter including introns as well as exons, a regulatory upstream or downstream sequence of a gene, a sequence involved in repairing a gene or in homologous recombination, a short sequence contained in a plasmid, or another large nucleic acid that mediates integration of plasmids or nucleic acids, for example, phage integrase or a "Sleeping Beauty" transposon, a DNA suitable for antisense regulation or as a transcription factor decoy, an oligonucleotide sequences useful as an adjuvant to boost a vaccine response, for example, a CpG-rich sequence, or a small interfering RNA (siRNA).

71. A complex as claimed in claim 69 or claim 70, wherein transcriptional and/or translational control elements for the nucleic acid are provided and the nucleic acid is optionally packed in a phage or vector.

72. A complex as claimed in any one of claims 65 to 71, wherein the nucleic acid component is DNA.

73. A complex as claimed in any one of claims 65 to 71, wherein the nucleic acid component is RNA.

74. A complex as claimed in claim 65, comprising a nucleic acid and a transfection mixture as claimed in any one of claims 51 to 63.

75. A complex as claimed in claim 74, wherein the nucleic acid is as claimed in any one of claims 69 to73.

76. A process for the production of a complex as claimed in any one of claims 65 to 73, which comprises admixing components (i), (ii), (iii) and (iv).

77. A process as claimed in claim 76, wherein the components are admixed in the following order: lipid component, peptide/polycationic nucleic acid-binding component, nucleic acid.

78. A process for producing a complex as claimed in claim 74 or claim 75, which comprises incorporating the nucleic acid with a mixture as claimed in any one of claims 51 to 63.

79. A complex as claimed in any one of claims 65 to 75, obtainable by a process as claimed in any one of claims 76 to 78.

80. A non-viral transfection complex that comprises

(i) a nucleic acid,

(iii) a polycationic nucleic acid-binding component, and

(iv) a peptide as claimed in any one of claims 1 to 34.

81. A complex as claimed in claim 80, wherein the nucleic acid is as defined in any one of claims 69 to 73, the polycationic nucleic acid-binding component is as defined in any one of claims 36 to 41, and optionally the peptide is as defined in any one of claims 35 to 46, or is a peptide derivative as claimed in any one of claims 47 to 50.

82. A process for the production of a complex as claimed in claim 80 or claim 81, which comprises admixing components (i), (iii) and (iv) in the following order: peptide/polycationic nucleic acid-binding component, nucleic acid.

83. A complex as claimed in claim 80 or claim 81, obtainable by a process as claimed in claim 82.

84. A viral vector that comprises a peptide as claimed in any one of claims 1 to 34.

85. A viral vector as claimed in claim 84, wherein the viral vector is a genetically engineered, replication-defective derivative of a retrovirus, a lentivirus, an adenovirus, an adeno-associated virus (AAV), or a herpes simplex virus (HSV).

86. A viral vector as claimed in claim 85, wherein the viral vector is an adenovirus.

87. A viral vector as claimed in claim 85, wherein the adenovirus is adenovirus type 5.

88. A viral vector as claimed in any one of claims 84 to 87, wherein the peptide is incorporated in a protein of the viral capsid or coat.

89. A viral vector as claimed in claim 87 or claim 88, wherein the peptide is incorporated in the HI region of the fibre protein in the adenoviral capsid.

90. A viral vector as claimed in any one of claims 84 to 87, wherein the peptide forms a complex with the vector, the peptide comprising a cationic domain that is capable of binding electrostatically to the viral capsid or coat.

91. A viral vector as claimed in claim 90, wherein the peptide is as claimed in any one of claims 35 to 46, or is in the form of a peptide derivative as claimed in any one of claims 47 to 50.

92. A viral vector as claimed in any one of claims 84 to 87, wherein the peptide incorporated with the viral vector by means of an antibody that is capable of binding to the virus.

93. A viral vector as claimed in claim 92, wherein the antibody is a bispecific antibody capable of binding to the peptide and to the virus.

94. A viral vector as claimed in claim 92, wherein the peptide and the antibody are in the form of a fusion protein.

95. A viral vector as claimed in any one of claims 92 to 94, wherein the antibody binds to an epitope on the viral capsid or coat.

96. A viral vector as claimed in any one of claims 92 to 95, wherein the antibody is of any antibody class, is an antigen-binding domain or domains, and/or is or is derived from a chimeric or humanised antibody.

97. A method of transfecting a cell with a nucleic acid, which comprises contacting the cell in vitro or in vivo with a transfection complex or a viral vector as claimed in any one of claims65 to 75, 79 to 81 and 83 to 96.

98. A pharmaceutical composition which comprises a transfection complex or a viral vector as claimed in any one of claims 65 to 75, 79 to 81 and 83 to 96, in admixture or conjunction with a pharmaceutically suitable carrier.

99. A method for the treatment or prophylaxis of a condition caused in human or in a non-human animal by a defect and/or a deficiency in a gene, which comprises administering a transfection complex or viral vector as claimed in any one of

claims 65 to 75, 79 to 81 and 83 to 96 to the human or to the non-human animal.

100. A method for therapeutic or prophylactic immunisation of a human or of a non-human animal, which comprises administering a transfection complex or viral vector as claimed in any one of claims 65 to 75, 79 to 81 and 83 to 96 to the human or to the non-human animal.

101. A method of anti-sense therapy, which comprises administering a transfection complex or viral vector as claimed in any one of claims 65 to 75, 79 to 81 and 83 to 96 to a human or to a non-human animal.

102. A transfection complex or viral vector as claimed in any one of claims 65 to 75, 79 to 81 and 83 to 96 for use as a medicament or a vaccine.

103. Use of a transfection complex or viral vector as claimed in any one of claims 65 to 75, 79 to 81 and 83 to 96 for the manufacture of a medicament for the prophylaxis of a condition caused in a human or a non-human animal by a defect and/or a deficiency in a gene, or for therapeutic or prophylactic immunisation, or for anti-sense therapy.

104. A kit that comprises

(i) nucleic acid,

(ii) a lipid component,

(iii) a polycationic nucleic acid-binding component, and

(iv) a peptide as claimed in any one of claims 1 to

105. A kit that comprises

(i) nucleic acid,

(iii) a polycationic nucleic acid-binding component, and

(iv) a peptide as claimed in any one of claims 1 to 30.

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106. A bispecific antibody that is capable of binding to a virus and to a peptide as claimed in any one of claims 1 to 34.

107. A fusion protein that comprises a peptide as claimed in any one of claims 1 to 34 and antibody that is capable of binding to a virus.

108. A method for identifying an siRNA, which comprises transfecting a cell that expresses a target gene with the siRNA and quantifying expression levels.

6.6.03

ABSTRACT

TRANSFECTION COMPLEXES

A peptide consisting of or comprising an amino acid sequence selected from

a) $PX^1X^2X^3T$ [SEQ.ID.NO.:1];
b) PSX^4S [SEQ.ID.NO.:2];
c) $QX^5X^6X^7Q$ [SEQ.ID.NO.:3];
d) SX^8S [SEQ.ID.NO.:4],

in which X^1, X^2 and X^3, which may be the same or different, each represents an amino acid residue;
X^4 represents an amino acid residue; and
X^5 and X^7, which may be the same or different, each represents an amino acid residue, X^6 represents an amino acid residue having an amide side chain; and
X^8 represent an amino acid having an aliphatic side chain, which peptide binds to dendritic cells and also to other types of celles. The peptide may be used a target non-viral and viral vectors to such cells.

6.6.03

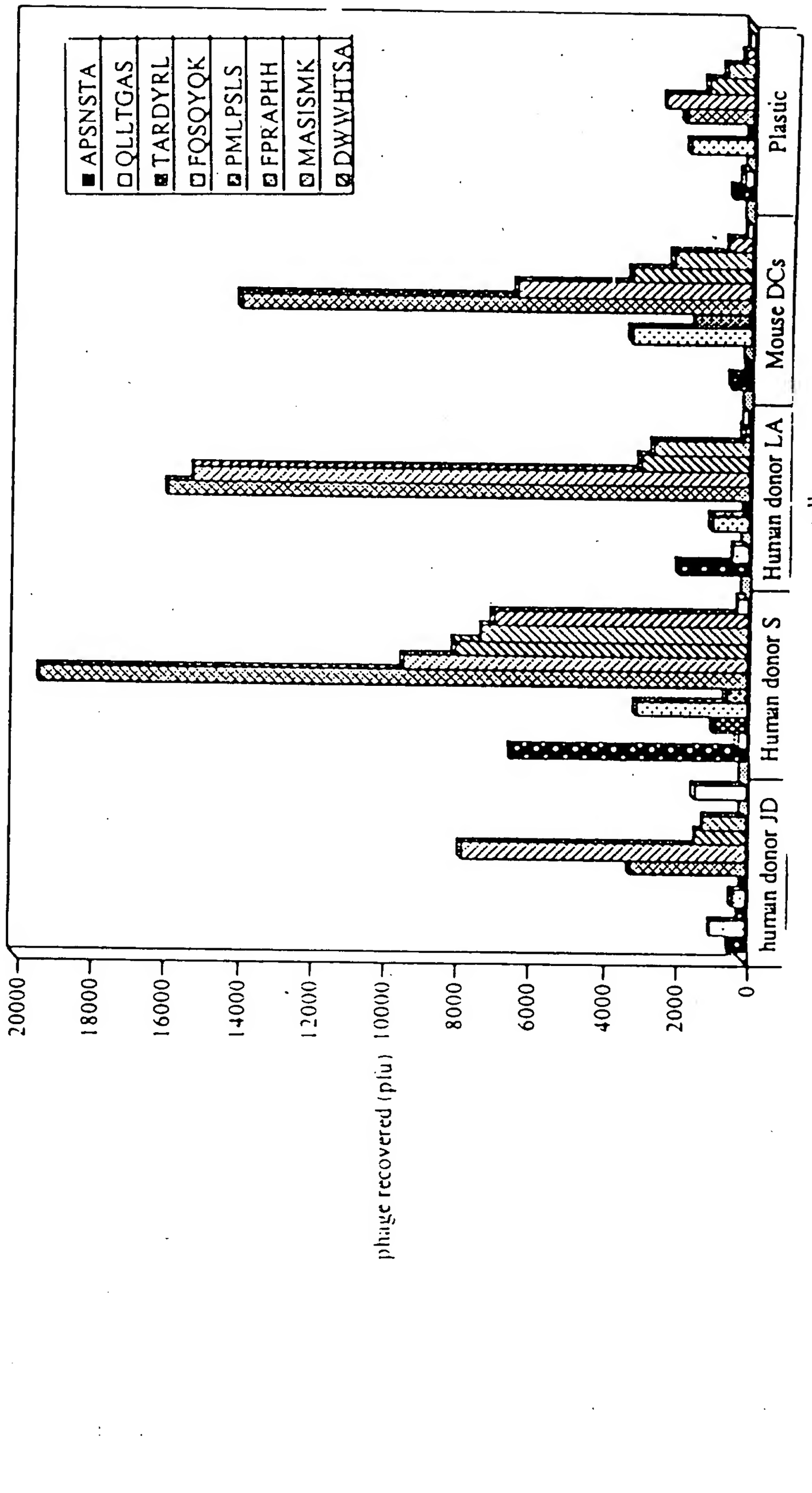

APSNSTA
QLLTGAS
TARDYRL
FQSQYQK
PMLPSLS
FPRAPHH
MASISMK
DWWHTSA
Plastic
Mouse DCs
Human donor LA
Human donor S
human donor JD
cells
phage recovered (plu)
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0

Figure 1

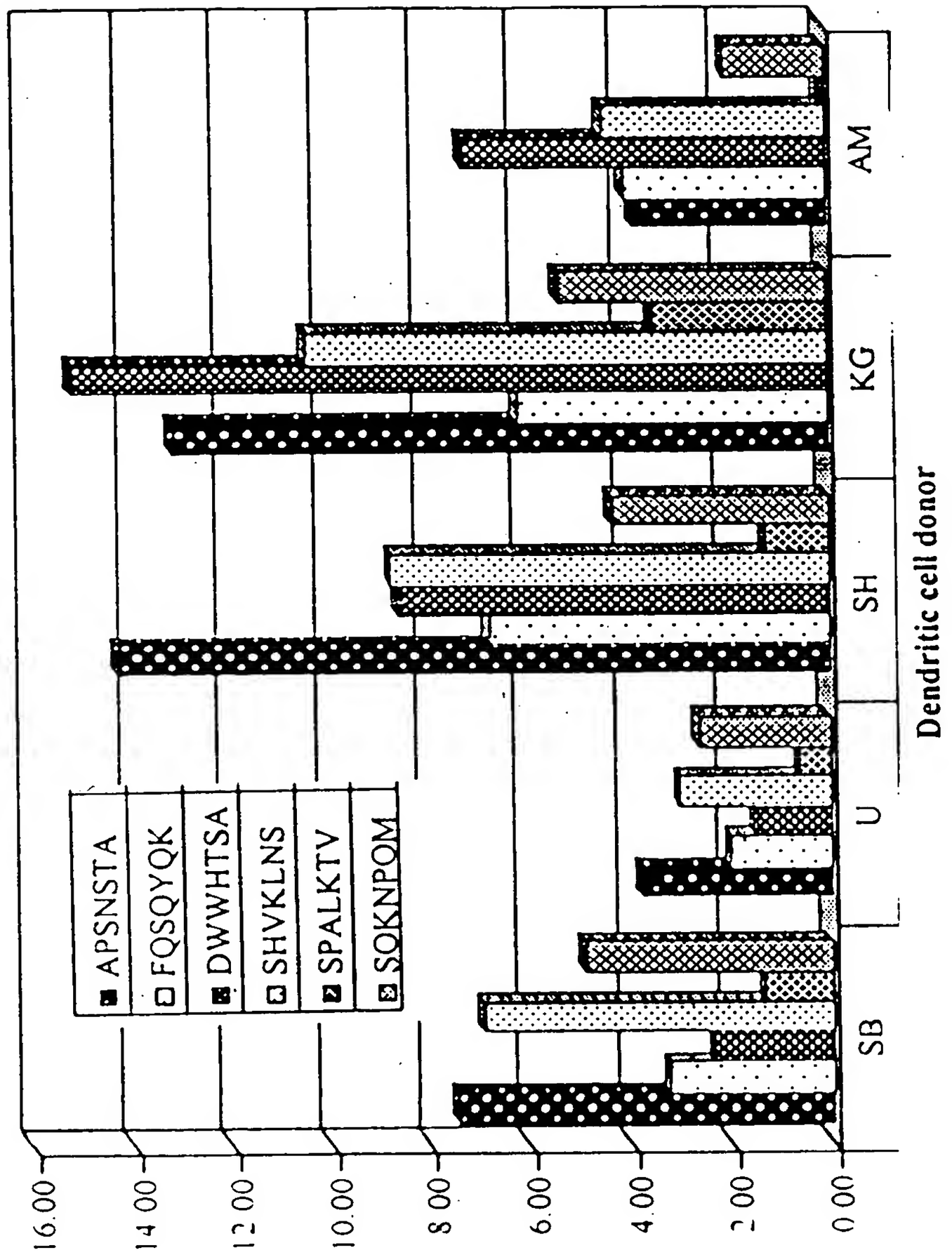

APSNSTA
FQSQYQK
DWWHTSA
SHVKLNS
SPALKTV
SQKNPQM
SB
U
SH
KG
AM
Dendritic cell donor
Percentage of cells positive relative to no insert phage
16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00

Figure 2

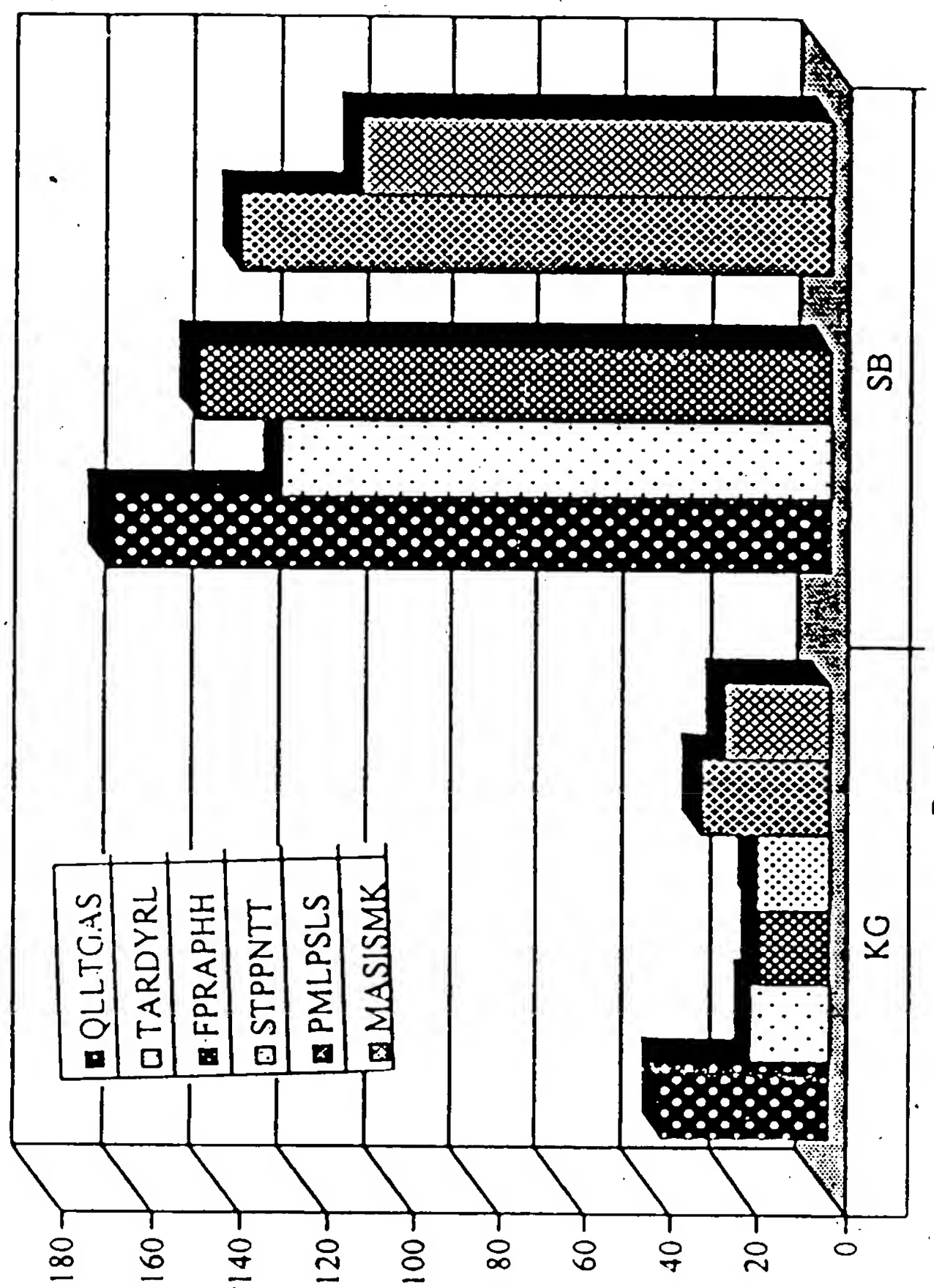

QLLTGAS
TARDYRL
FPRAPHH
STPPNTT
PMLPSLS
MASISMK
SB
KG
Dendritic cell donor
Percentage positive cells relative to no insert phage
180
160
140
120
100
80
60
40
20
0

Figure 3

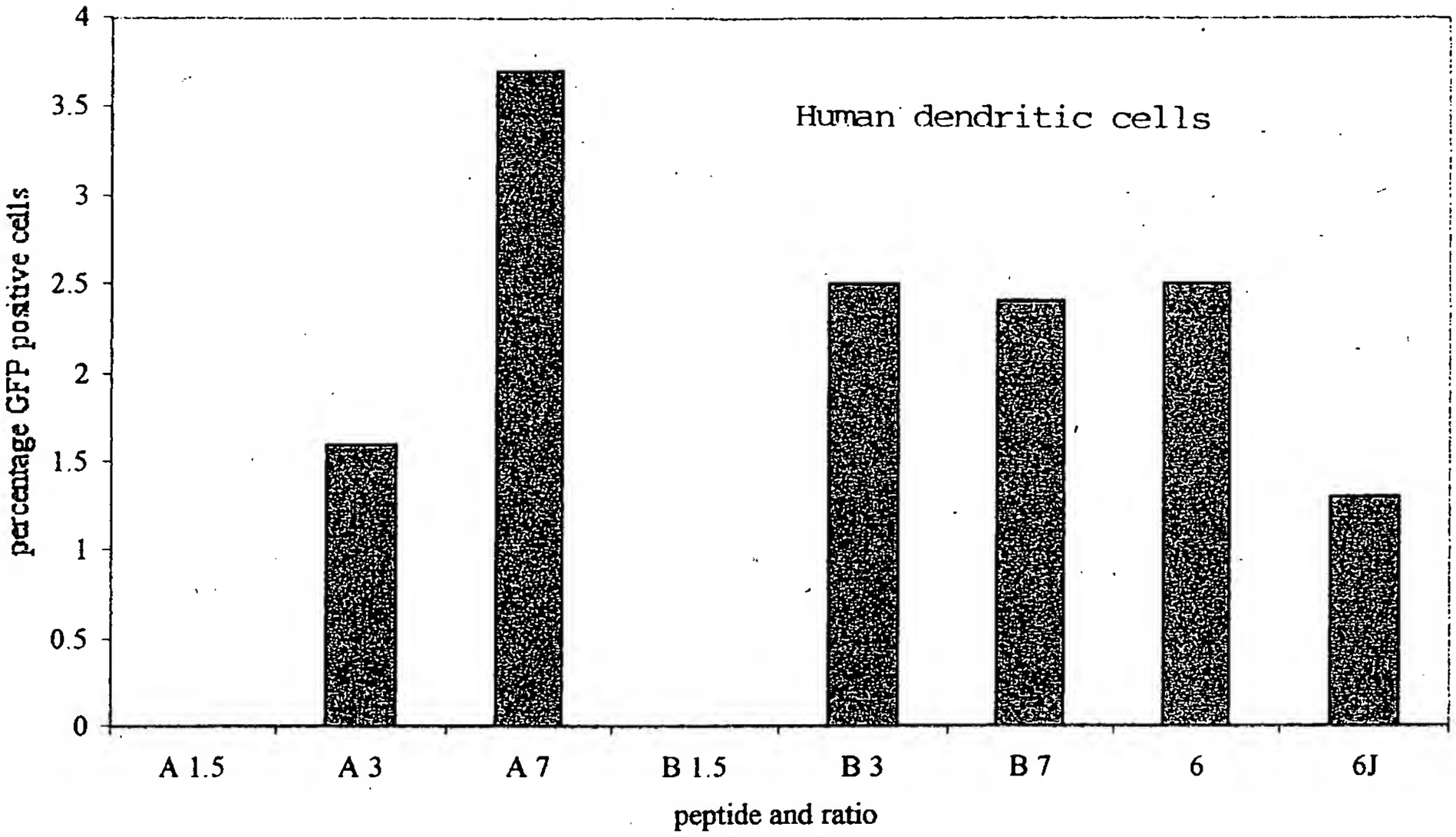


Figure 4

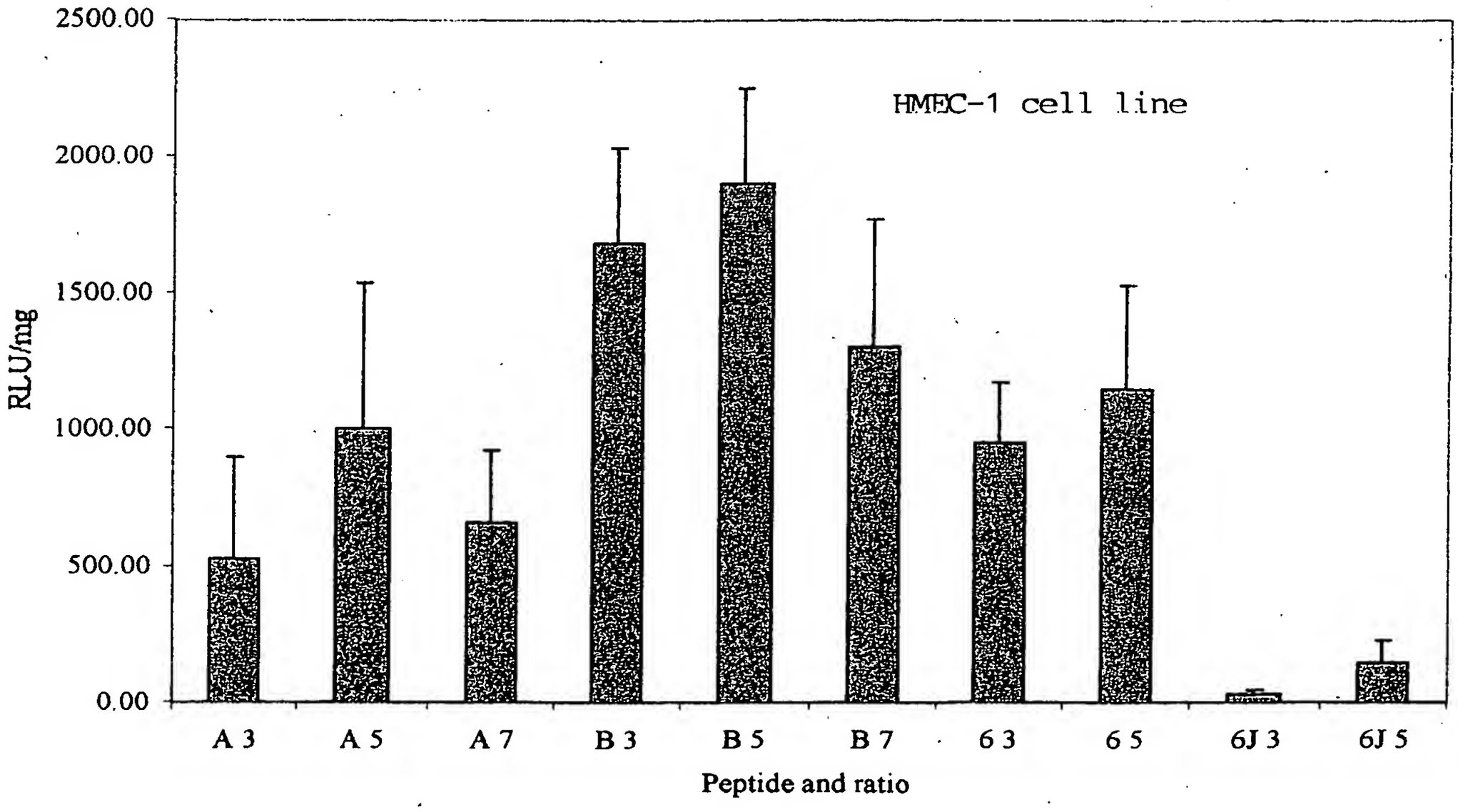
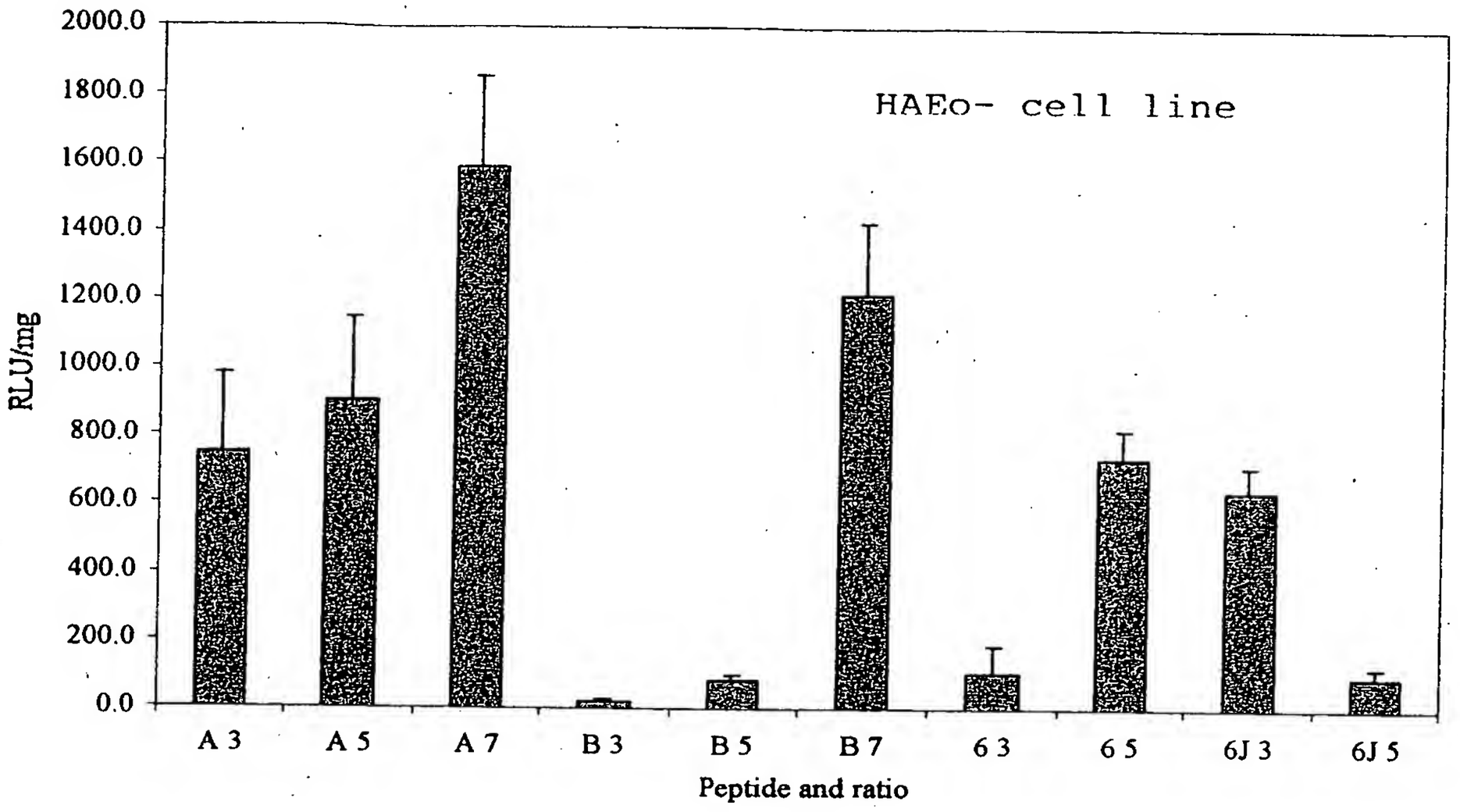


Figure 5



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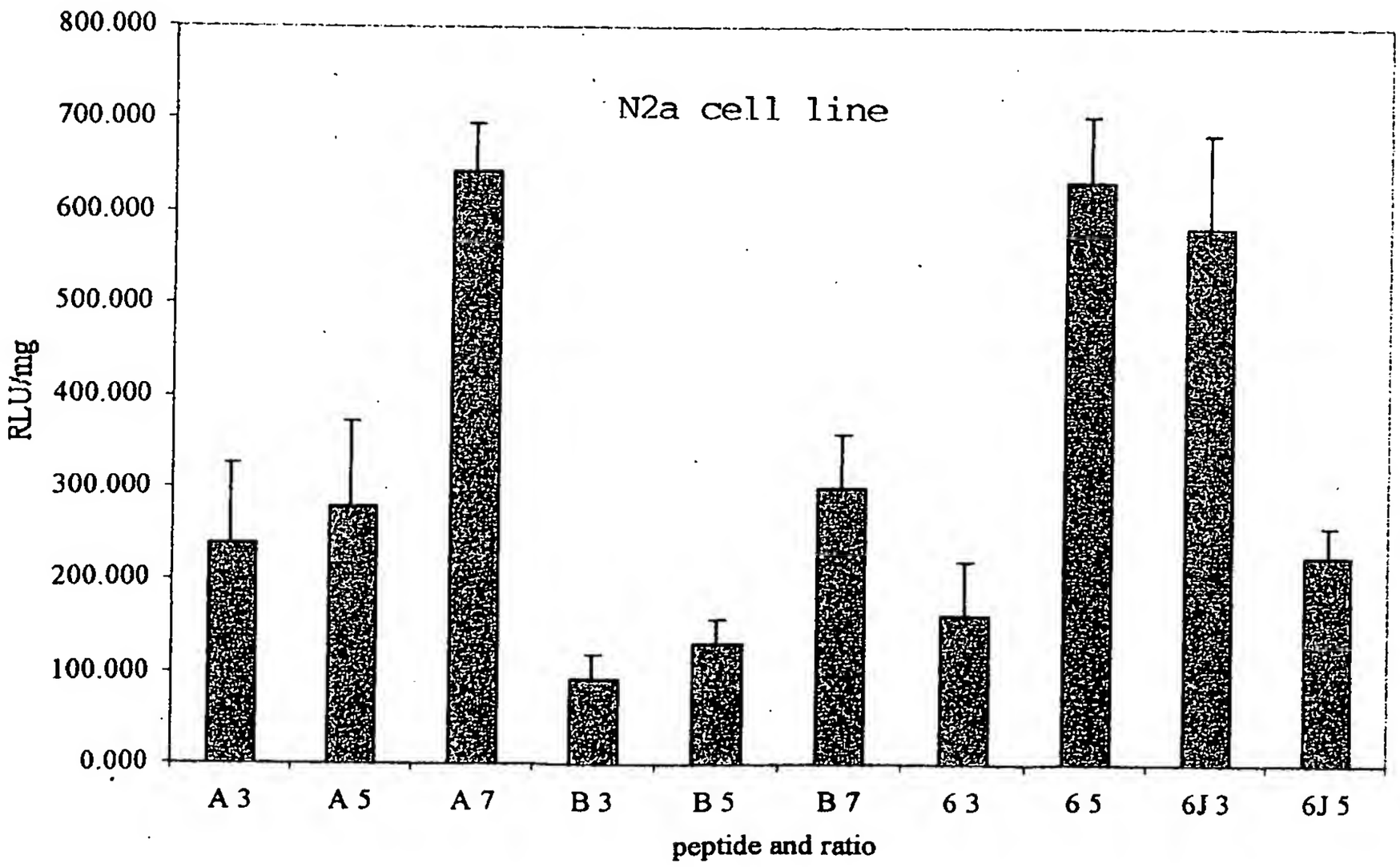

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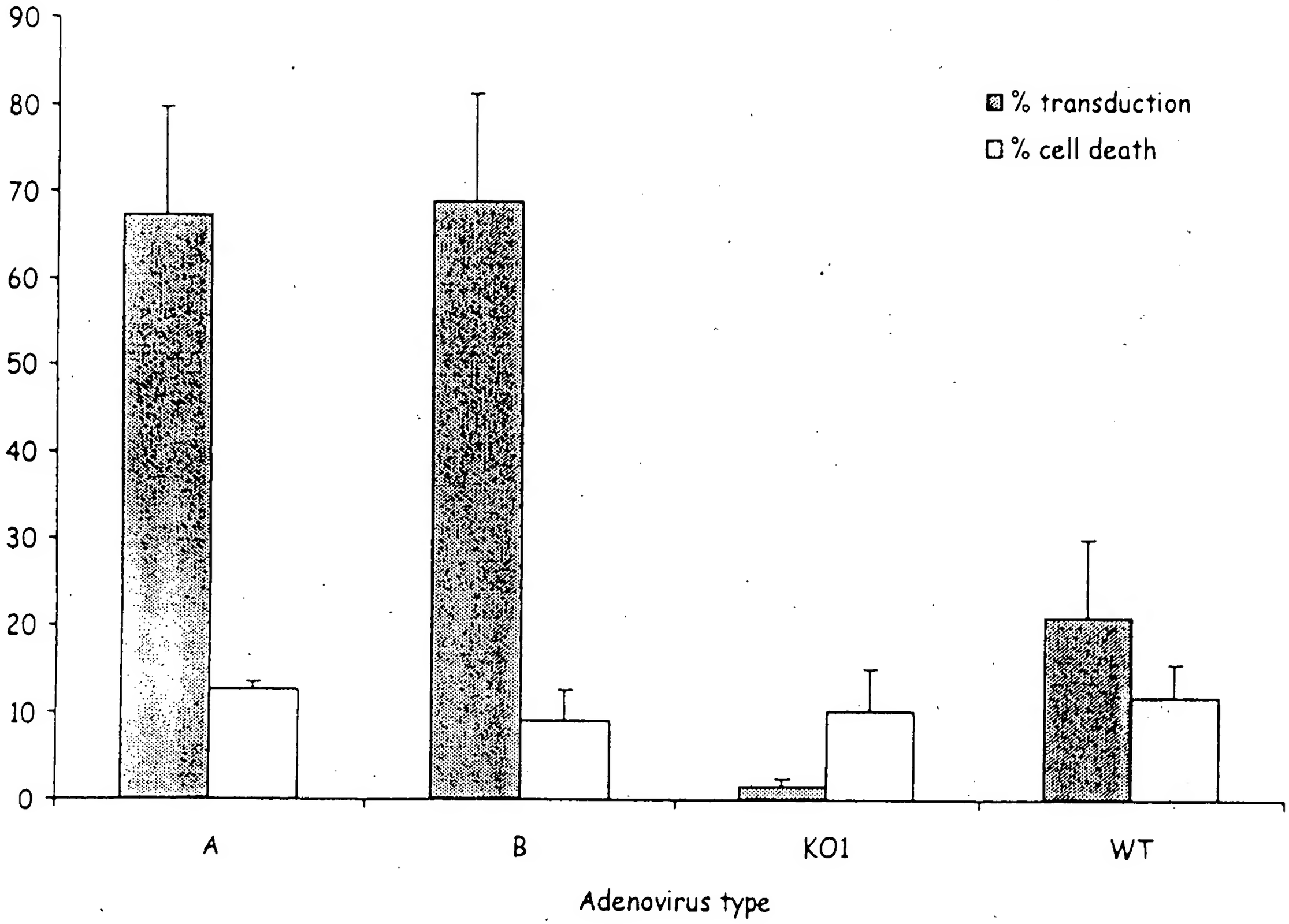
90
80
70
60
50
40
30
20
10
0
% transduction
% cell death
A
B
KO1
WT
Adenovirus type

Figure 8